Characterization of Dickeya solani strains and identification of bacterial and plant signals involved in induction of virulence

Malgorzata Golanowska

Intercollegiate Faculty of Biotechnology
University of Gdansk and Medical
University of Gdansk,
Department of Biotechnology,
Laboratory of Plant Protection
and Biotechnology

Institut National
des Sciences Apliquées de Lyon
Laboratoire de Microbiologie, Adaptation
et Pathogénie

2015ISAL0087

Malgorzata Golanowska
Life Sciences and Mathematics Interdisciplinary Doctoral Studies;
Ecole Doctorale: Evolution, Ecosystèmes, Microbiologie, Modélisation

Characterization of *Dickeya solani* strains and identification of bacterial and plant signals involved in the induction of virulence.

Caractérisation de souches de *Dickeya solani* et identification de signaux bactériens ou végétaux impliqués dans l'induction de gènes de virulence.

Thesis presented to Scientific Board of Intercollegiate Faculty of Biotechnology UG MUG
and the Institute National des Sciences Apliquees de Lyon
in order to obtain the degree of doctor in biological sciences in the field of biochemistry

Supervisors:

Prof. Dr hab. Ewa ŁOJKOWSKA
Intercollegiate Faculty of Biotechnology
University of Gdansk & Medical University of Gdansk, POLAND

Dr. Nicole HUGOUVIEUX-COTTE-PATTAT
Laboratory of Microbiology Adaptation and Pathogenesis (UMR 5240)
CNRS, Université Lyon 1 & INSA de Lyon, FRANCE

Gdańsk 2015

INSA Direction de la Recherche - Ecoles Doctorales – Quinquennal 2011-2015

SIGLE	ECOLE DOCTORALE	NOM ET COORDONNEES DU RESPONSABLE
CHIMIE	CHIMIE DE LYON **http://www.edchimie-lyon.fr** Sec : Renée EL MELHEM Bat Blaise Pascal 3e etage 04 72 43 80 46 Insa : R. GOURDON secretariat@edchimie-lyon.fr	M. Jean Marc LANCELIN Université de Lyon – Collège Doctoral Bât ESCPE 43 bd du 11 novembre 1918 69622 VILLEURBANNE Cedex Tél : 04.72.43 13 95 directeur@edchimie-lyon.fr
E.E.A.	ELECTRONIQUE, ELECTROTECHNIQUE, AUTOMATIQUE **http://edeea.ec-lyon.fr** Sec : M.C. HAVGOUDOUKIAN Ecole-doctorale.eea@ec-lyon.fr	M. Gérard SCORLETTI Ecole Centrale de Lyon 36 avenue Guy de Collongue 69134 ECULLY Tél : 04.72.18 60.97 Fax : 04 78 43 37 17 Gerard.scorletti@ec-lyon.fr
E2M2	EVOLUTION, ECOSYSTEME, MICROBIOLOGIE, MODELISATION **http://e2m2.universite-lyon.fr** Sec : Safia AIT CHALAL Bat Atrium- UCB Lyon 1 04.72.44.83.62 Insa : S. REVERCHON Safia.ait-chalal@univ-lyon1.fr	M. Fabrice CORDEY Laboratoire de Géologie de Lyon Université Claude Bernard Lyon 1 Bât Géode – Bureau 225 43 bd du 11 novembre 1918 69622 VILLEURBANNE Cédex Tél : 04.72.44.83.74 Sylvie.reverchon-pescheux@insa-lyon.fr fabrice.cordey@ univ-lyon1.fr
EDISS	INTERDISCIPLINAIRE SCIENCES-SANTE **http://www.ediss-lyon.fr** Sec : Safia AIT CHALAL Bat Atrium – UCB Lyon 1 04 72 44 83 62 Insa : Safia.ait-chalal@univ-lyon1.fr	Mme Emmanuelle CANET-SOULAS INSERM U1060, CarMeN lab, Univ. Lyon 1 Bâtiment IMBL 11 avenue Jean Capelle INSA de Lyon 696621 Villeurbanne Tél : 04.72.11.90.13 Emmanuelle.canet@univ-lyon1.fr
INFOMATHS	INFORMATIQUE ET MATHEMATIQUES **http://infomaths.univ-lyon1.fr** Sec :Renée EL MELHEM Bat Blaise Pascal 3e etage infomaths@univ-lyon1.fr	Mme Sylvie CALABRETTO LIRIS – INSA de Lyon Bat Blaise Pascal 7 avenue Jean Capelle 69622 VILLEURBANNE Cedex Tél : 04.72. 43. 80. 46 Fax 04 72 43 16 87 Sylvie.calabretto@insa-lyon.fr
Matériaux	MATERIAUX DE LYON **http://ed34.universite-lyon.fr** Sec : M. LABOUNE PM : 71.70 –Fax : 87.12 Bat. Direction 1er et. Ed.materiaux@insa-lyon.fr	M. Jean-Yves BUFFIERE INSA de Lyon MATEIS Bâtiment Saint Exupéry 7 avenue Jean Capelle 69621 VILLEURBANNE Cedex Tél : 04.72.43 71.70 Fax 04 72 43 85 28 Ed.materiaux@insa-lyon.fr
MEGA	MECANIQUE, ENERGETIQUE, GENIE CIVIL, ACOUSTIQUE **http://mega.universite-lyon.fr** Sec : M. LABOUNE PM : 71.70 –Fax : 87.12 Bat. Direction 1er et. mega@insa-lyon.fr	M. Philippe BOISSE INSA de Lyon Laboratoire LAMCOS Bâtiment Jacquard 25 bis avenue Jean Capelle 69621 VILLEURBANNE Cedex Tél : 04.72 .43.71.70 Fax : 04 72 43 72 37 Philippe.boisse@insa-lyon.fr
ScSo	ScSo* **http://recherche.univ-lyon2.fr/scso/** Sec : Viviane POLSINELLI Brigitte DUBOIS Insa : J.Y. TOUSSAINT viviane.polsinelli@univ-lyon2.fr	Mme Isabelle VON BUELTZINGLOEWEN Université Lyon 2 86 rue Pasteur 69365 LYON Cedex 07 Tél : 04.78.77.23.86 Fax : 04.37.28.04.48 isavonb@dbmail.com

The author has performed the work within the project funded from the Human Capital Programme entitled: “We educate the best – comprehensive career advancement programme for PhD students, young doctors and academic staff of the University of Gdańsk”, under Task 2: “Life Science and Mathematics Interdisciplinary Doctoral Studies”. (Decision number: UDA-POKL.04.01.01-00-017/1000).

HUMAN CAPITAL
NATIONAL COHESION STRATEGY

EUROPEAN
SOCIAL FUND

The work was co-funded by Polish National Science Centre based on decision no.: UMO-2013/08/M/NZ9/00974

NATIONAL SCIENCE CENTRE
POLAND

The author has obtained a doctoral stipend from The French Ministry of Foreign Affairs The Eiffel Excellence Scholarship Program, decision no: UR / EIFFEL-DOCTORAT 2013 / 783962F

MINISTÈRE DES AFFAIRES ÉTRANGÈRES
CAMPUS FRANCE
campusfrance.org

This research work was supported by the system project “InnoDoktorant – Scholarships for PhD students, VIth edition”. Project is co-financed by the European Union in the frame of the European Social Fund.

HUMAN CAPITAL
NATIONAL COHESION STRATEGY

POMORSKIE
VOIVODESHIP

EUROPEAN
SOCIAL FUND

Pracę dedykuję mojej Rodzinie.
To my Family.

Dear Prof. Dr hab. Ewa Łojkowska ,
I wanted to thank you for the opportunity to perform my dissertation in the Laboratory of Plant Protection and Biotechnology under your supervision and for your support, openness, support and understanding.

Dear Dr. Nicole Hugouvieux-Cotte-Pattat,
I would like to thank you for the opportunity to perform a part of my study in your Laboratory and for hospitality and support.

Dear Colleagues from IFB UG-MUG and MAP, I would like to thank you for the opportunity to work with you and great atmosphere and encouraging conversations.

“Reductionist experiments are powerful, but the lure of their yes/no results can keep us from doing discovery experiments that may be complicated and messy, but also more biologically realistic and practically relevant. Familiar examples include studies focusing on single genes rather than multigenic traits, model systems instead of natural hosts, sterile potting mix in place of natural soil, seedlings rather than mature plants, and controlled rather than field environments. Every researcher struggles to balance experimental feasibility with biological meaning, but a convenient and familiar assay can give deceptive results that hide a more interesting truth.”

Allen, C., Bent, A., and Charkowski, A. 2009. Underexplored niches in research on plant pathogenic bacteria. Plant Physiol. 150:1631-1637

Content

List of tables:

List of figures:

1 Abstracts

1.1 Summary in English

Pectinolytic bacteria from the genera *Pectobacterium* (former *Erwinia carotovora*) and *Dickeya* (former *Erwinia chrysanthemi*) are casual agents of blackleg and soft rot diseases. They cause crop damages and high economic losses. For example, losses caused by pectinolytic bacteria are evaluated at about 2 to 10% of the potato yield, depending on the year. In 2009, the value of potato losses in Europe was estimated to reach 250 million Euros. During the last years, *Dickeya* strains have been more and more often isolated from diseased plants in Poland, France and other European countries. The genus *Dickeya* is a highly diverse group, which according to the present nomenclature contains seven species: *D. aquatica, D. chrysanthemi, D. dadantii, D. dianthicola, D. paradisiaca, D. solani* and *D. zeae*. Recent results, obtained in different European countries, indicate that a new species – *Dickeya solani* can efficiently infect potato plants and cause disease symptoms in temperate climate. The *D. solani* strains are considered as more aggressive than other blackleg causing bacteria. Preliminary analysis suggested that they need lower optimal temperatures for disease development as well as lower inoculum levels for infection spreading. They seem to have a greater ability to colonize potato plants roots and to spread through the plants' vascular system. *D. solani* strains produce a wide range of plant cell-wall degrading enzymes which are the main virulence factors.

The aims of the study were: 1) phenotypic and genotypic characterization of the *D. solani* strains isolated in countries with different climatic conditions: Poland, Finland and Israel; 2) study of the potato tuber extract influence on the expression of selected *D. solani* genes: *pelD, pelL, tssK, lfaA;* 3) comparative genomics of ten *D. solani* strains, performed on 4 genomes sequenced for this study and 6 genome sequences available in the GenBank databases.

The results showed that the strains from different climatic conditions have identical rep-PCR profiles (three different primers sets were used – ERIC, REP and BOX) and Restriction Fragments Length Polymorphism-Pulse Field Gel Electrophoresis (RFLP – PFGE) profiles, but they do differ phenotypically, especially in the activity of plant cell-wall degrading enzymes. Polish strains have higher activities of pectinolytic, cellulolytic and proteolytic enzymes than Finnish and Israeli strains.

D. solani mutants in the *pelD, pelL, tssK, lfaA* genes were obtained by the means of site-specific mutagenesis. The highest induction by plant extracts was observed for the *lfaA* gene. The *pelL* expression was also induced by plant derived signal(s), contrary to *pelD* and *tssK*.

Comparative genomics analysis has elucidated the pangenome shape of 10 *D. solani* strains. Ten *D. solani* genomes are encoding 41 947 proteins. The *D. solani* genes form 10 analysed genomes were grouped into 5 045 Orthologous Groups, 3 809 belonging to the core genome, 413 to the accessory genome and 823 to the unique genome. The analysis of the protein sequences of pathogenicity-related genes (9 cell wall – degrading enzymes) as well as their 19 regulators (chosen on the basis of knowledge available for *D. dadantii* 3937, the most studied *Dickeya* strain belonging to a closely related species) showed 100 % sequence homology within the 10 genomes.

All genenomic studies proved that *D. solani* strains form a very homogenous group. On the other hand, the phenotypic analysis showed some variability among strains from different climatic conditions. The key to the observed variations in the phenotypic traits can be a different regulation of the expression of the genes encoding virulence factors, which can be influenced by temperature, pH, iron deprivation or oxygen and nitrogen availability, as well as the presence of specific plant tissue compounds.

1.2 Summary in French

Les bactéries pectinolytiques des genres *Pectobacterium* (ancien nom *Erwinia carotovora*) et *Dickeya* (ancien nom *Erwinia chrysanthemi*) sont les agents des maladies de la jambe noire et de la pourriture molle. Ils provoquent des dommages aux cultures et des pertes économiques élevées. Les pertes causées par les bactéries pectinolytiques sont évaluées à environ 2 à 10% du rendement de pommes de terre, en fonction de l'année. En 2009, les pertes en pommes de terre en Europe ont été estimées à 250 millions d'euros. Au cours des dernières années, des souches de *Dickeya* ont été de plus en plus souvent isolées de plantes malades en Pologne, en France et d'autres pays européens.

Le genre *Dickeya* est un groupe très diversifié, qui, selon la nomenclature actuelle contient sept espèces: *D. aquatica, D. chrysanthemi, D. dadantii, D. dianthicola, D. paradisiaca, D. solani et D. zeae*. Les résultats récents, obtenus dans différents pays européens, indiquent qu'un nouveau groupe de souches de *Dickeya* peut infecter efficacement les plantes de pomme de terre et causer des symptômes de la maladie en climat tempéré. Les souches de *D. solani* sont considérés comme plus agressives que les autres bactéries causant la jambe noire. Une analyse préliminaire a suggéré qu'elles ont besoin de plus faibles températures optimales pour le développement de la maladie ainsi que de niveaux d'inoculum inférieurs pour la propagation de l'infection. Elles semblent avoir une plus forte capacité à coloniser les racines de plantes de pomme de terre et à se propager à travers le système vasculaire de la plante. Les souches de *D. solani* produisent une large gamme d'enzymes dégradant de la paroi cellulaire végétale, qui sont les principaux facteurs de virulence.

Les objectifs de l'étude étaient les suivants: 1) la caractérisation phénotypique et génotypique des souches de *D. solani* isolées dans des pays ayant des conditions climatiques différentes: Pologne, Finlande et Israël, 2) l'étude de l'influence d'extraits de pomme de terre sur l'expression de quelques gènes sélectionnés de *D. solani: pelD, pelL, tssk, lfaA*, 3) la génomique comparative de dix souches de *D. solani*, basée sur 4 génomes séquencés pour cette étude et 6 séquences génomiques disponibles dans la base de données GenBank.

Les résultats ont montré que les souches isolées de différentes conditions climatiques ont des profils identiques en rep-PCR (trois amorces différentes ont été utilisées – ERIC, REP,BOX) et en RFLP-PFGE, mais ils diffèrent phénotypiquement, en particulier dans l'activité des enzymes dégradant la paroi cellulaire végétale. Les souches polonaises ont des activités plus élevés que les souches finlandaises et israéliennes pour les enzymes pectinolytique, protéolytiques et cellulolytiques.

Des mutants de *D. solani* dans les gènes *pelD, pelL, tssk, lfaA* ont été obtenus par mutagenèse spécifique. La plus forte induction par des extraits de plantes a été observée pour le gène *lfaA*. L'expression de *pelL* est également induite par des signaux dérivés de la plante, contrairement à *pelD* et *tssk*.

L'analyse de génomique comparative a élucidé la structure du pangénome de 10 souches de *D. solani*. Ces 10 génomes codent 41 947 protéines qui ont été classées en 5 045 groupes orthologues, 3 809 appartenant au génome cœur, 413 au génome accessoire et 823 spécifiques de génome unique. Des gènes liés à la pathogénicité, ainsi que leurs régulateurs ont été choisis sur la base des connaissances disponibles pour *D. dadantii* 3937, la souche la plus étudiée de *Dickeya* appartenant à une espèce proche. Une analyse de leur séquence protéique n'a montré aucune différence dans les 10 souches.

En conclusion, toutes les études génomiques ont montré que les souches de *D. solani* forment un groupe très homogène. Cependant, leur analyse phénotypique révèle une certaine variabilité entre les souches provenant de différentes conditions climatiques. La raison des variations observées dans les traits phénotypiques peut être liée à la régulation de l'expression des gènes codant les facteurs de virulence qui peuvent être influencés par la température, le pH, la carence en fer ou en oxygène et la disponibilité en azote, ainsi que par la présence de composés spécifiques des tissus végétaux.

1.3 Summary in Polish

Bakterie pektynolityczne z rodzajów *Pectbacterium* (dawniej *Erwinia carotovora*) i *Dickeya* (dawniej *Erwinia chrysanthemi*) powodują choroby takie jak czarna nóżka oraz mokra zgnilizna. Choroby te, w zależności od warunków klimatycznych, powodują straty od 2 do 10% plonów. W 2009 roku wartość strat plonów ziemniaka, spowodowanych przez bakterie pektynolityczne w Europie, została oszacowana na 250 milionów Euro. W ciągu ostatnich kilku lat, szczepy bakterii z rodzaju *Dickeya* były coraz częściej izolowane z chorych tkanek roślinnych w wielu krajach europejskich. Rodzaj *Dickeya* jest wysoko zróżnicowaną grupą bakterii, która zgodnie z obowiązującą klasyfikacją obejmuje siedem gatunków: *D. aquatica*, *D. chrysanthemi*, *D. dadantii*, *D. dianthicola*, *D. paradisiaca*, *D. solani* oraz *D. zeae*. Ostatnie doniesienia z wielu krajów europejskich wskazują, że bakterie należące do ustanowionego w 2014 roku gatunku *D. solani* mogą efektywnie infekować i wywoływać symptomy chorobowe na roślinach ziemniaka w strefie klimatu umiarkowanego. Szczepy *D. solani* są bardziej agresywne niż bakterie z gatunku *D. dianthicola*. Przeprowadzone badania wykazały, że mają zdolność do infekowania roślin w temperaturze typowej dla klimatu umiarkowanego a także iż stosunkowo niski poziomu inokulum bakteryjnego może wywoływać objawy chorobowe.

Wykazano także, iż bakterie z gatunku *D. solani* mają lepsza zdolność do kolonizacji korzeni roślin ziemniaka a także rozprzestrzeniania się poprzez tkankę naczyniową roślin. *D. solani*, podobnie jak inne bakterie pektynolityczne wytwarza szerokią gamę zakres enzymów degradujących składniki ścian komórkowych roślin.

Celem przedstawionej rozprawy doktorskiej była: 1) fenotypowa oraz genotypowa charakterystyka szczepów *D. solani* pochodzących z różnych stref klimatycznych, izolowanych w takich krajach jak: Polska, Finlandia oraz Izrael; 2) zbadanie wpływu ekstraktu otrzymanego z bulw ziemniaka na ekspresję w komórkach *D. solani* wybranych genów : *pelD, pelL, tssK, lfaA*; 3) genomika porównawcza dziesięciu szczepów *D. solani*, przeprowadzona na sekwencjach genomowych czterech szczepów sekwencjonowaniach w ramach niniejszej pracy i różniących się poziomem wirulencji, oraz na sekwencjach genomowych sześciu szczepów *D. solani* dostępnych w GenBank.

Wyniki analizy geno- oraz fenotypowej wykazały, że szczepy pochodzące z różnych warunków klimatycznych posiadają identyczne profile rep-PCR (badania przeprowadzono

z użyciem starterów REP, ERIC oraz BOX) a także identyczne profile restrykcyjne uzyskane w analizie przeprowadzonej metodą elektroforezy pulsowej (RFLP-PFGE), ale różnią się cechami fenotypowymi, w szczególności w poziomem aktywności enzymów degradujących ściany komórkowe. Szczepy *D. solani* wyizolowane w Polsce wykazywały wyższą aktywność pektynaz, celulaz oraz proteaz niż szczepy wyizolowane w Finlandii czy Izraelu.

W kolejnym etapie pracy do genów kodujących czynniki odgrywające istotną rolę w rozwoju infekcji wprowadzono kasety z genem reporterowym GUS, w celu sprawdzenia wpływu ekstraktu otrzymanego z bulw ziemniaka na ekspresję zmutowanych genów.

Przeprowadzone badania wykazały, iż ekstrakt tkankowy najefektywniej induje ekspresję genu *lfaA*. Ekspresja genu *pelL* była rozwnież indukowana przez ekstrakt, jednak poziom indukcji był niższy niż w pryzpadku genu *lfaA*. Natomiast ekspresja genów *pelD* oraz *tssK* nie były indukowane przez ekstrakt roślinny.

Analiza porównawcza genomów 10 szczepów bakterii *D. solani* pozwoliła na opisanie pangenomu tego gatunku. W genomach 10 szczepów *D. solani* zidentyfikowano 41 947 genów. Geny te zostały zgrupowane w 5 045 grup ortologicznych (ang. Ortologous Groups); 3 809 należy do tzw. rdzenia genomu (ang. core genome), 413 do genomu dodatkowego (ang. accessory genome) a 823 to tzw. genomu unikatowego (ang. unique genome). Analiza sekwencji białkowych dziewięciu enzymów degradujących składniki ścian komórkowych oraz dziewiętnastu regulatorów ekspresji genów kodujących wspomniane białka (wybranych na podstawie badań *D. dadantii* 3937), wykazała 100 % homologii sekwencji badanych białekw obrębie 10 analizowanych genomów *D. solani*.

Wykonane w ramach niniejszej rozprawy badania wykazały, iż szczepy z gatunku *D. solani* tworzą bardzo jednolitą genetycznie grupę bakterii. Z drugiej strony, badane szczepy wykazały pewne zróżnicowanie cech fenotypowych. Postawiono hipotezę, iż kluczem do obserwowanych różnic fenotypowych jest zróżnicowana, iż regulacja ekspresji genów kodujących czynniki wirulencji. Na różnice w ekspresji genów kodujących czynniki wirulencji mogą wpływać takie czynniki jak na przykład: temperatura, anaerobioza, pH środowiska, ograniczenie dostępności żelaza a także obecność różnego rodzaju związków pochodzenia roślinnego.

2 List of abbreviations

AHL	acyl-homoserine lactone
ANOVA	Analysis Of Variance
approx.	approximately
bp	base pair
CBD	cellulose-binding domain
CD	catalytic domain
CFU	colony forming unit
CMC	carboxymethylcellulose
CRP	cAMP receptor protein
CVP	crystal violet pectate
DNA	deoxyribonucleic acid
dNTP's	deoxynucleotide triphosphates
DKI	5-keto-4-deoxyuronate
Dsp	*Dickeya* spp.
Eca	*Erwinia carotovora*
Ech	*Erwinia chrysanthemi*
EDTA	Ethylenediaminetetraacetic acid
EG	endoglucanase
EPS	Exopolysacharide
ERIC	Enterobacterial Repetitive Intergenic Consensus
GUS	β-glucuronidase
IM	inner membrane
KDG	2-keto-3-deoxygluconate
LA	Luria Agar
LB	Luria Broth
LR	linker region
LS-BSR	Large scale Blast Score Ratio
min	minute
MLSA	Multi Locus Sequence Analysis
OCA	o-coumaric acid
OD	optical density
OG	Orthologous Group
ORF	Open Reading Frame
OM	outer membrane

Pba	*Pectobacterium atrosepticum*
Pcc	*Pectobacterium carotovorum* subspecies *carotovorum*
PCA	p-couramic acid
PCWDE	plant cell-wall degrading enzymes
PCR	Polymerase Chain Reaction
PE	potato tuber extract
Peh	polygalaturonase
Pel	pectate lyase
PFGE	Pulse Field Gel Electrophoresis
PG	polygalacturonate
PGA	polygalacturonic acid
Pme	pectin methyl-estrerase
PNP	p-nitrophenol
PNPU	p-nitrophenyl-β- D-glucuronid
PL	pectate lyase activity
PSB	dry weight of bacteria
Pwa	*Pectobacterium wasabiae*
REP	Repetitive Extragenic Palindromic
RFLP	restriction fragment length polymorphism
RGI	rhamnogalacturonan I
rpm	revolutions per minute
RTX	repeats in toxin
SRE	Soft Rot Enterobacteriaceae
TCA	t-coumaric acid
TS	Type Strain
TSA	tryptone soya agar
TSB	tryptone soya broth
T1SS	Type I Secretion System
T2SS	Type II Secretion System
T3SS	Type III Secretion System
T4SS	Type IV Secretion System
T5SS	Type V Secretion System
T6SS	Type VI Secretion System
UP	unsaturated products
Vfm	Virulence factor regulating
v/v	volume/volume

3 Introduction

Pectinolytic bacteria from the genera *Pectobacterium* (former *Erwinia carotovora, Eca*) and *Dickeya* (former *Erwinia chrysanthemi, Ech*) are casual agents of blackleg and soft rot diseases (Perombelon and Kelman 1980; Toth et al., 2003). They are in a group of top ten bacterial pathogens causing great damage to different crops (Mansfield et al., 2012). For example, potato yield reduction caused by *Dickeya* spp. was evaluated as about 20 to 25% in Israel (Tsror et al., 2009). During recent years, *Dickeya* spp. strains have been more and more often isolated from diseased plants in Poland, Finland, France, the Netherlands, Switzerland, other European countries and Israel (Laurila et al., 2008; Tsror et al., 2009; Sławiak et al., 2009ab; Toth et al., 2011).

The genus *Dickeya* is a highly diverse group, which according to the present nomenclature contains seven species: *Dickeya aquatica, Dickeya chrysanthemi, Dickeya dadantii, Dickeya dianthicola, Dickeya paradisiaca, Dickeya solani* and *Dickeya zeae* (Samson et al., 2005; Brady et al., 2012; Van der Wolf et al., 2014b; Parkinson et al., 2014). Recent results, obtained in different European countries, indicate that a new group of *Dickeya* spp. strains can efficiently infect potato plants and cause disease symptoms in temperate climate (Toth et al., 2011). This new group of *Dickeya* spp. strains – *D. solani* was already proposed in 2009, but established only in 2014, because it is genetically different group from other *Dickeya* (Slawiak et al., 2009b; Van der Wolf et al., 2014b). The *D. solani* strains are considered as more aggressive than other blackleg causing bacteria. The performed analysis suggested that the Type Strain of *D. solani* - IPO 2222 needs lower optimal temperatures for disease development as well as lower inoculum levels for infection spreading. *D. solani* IPO 2222 also has a greater ability to colonize potato plants roots and to spread through the plants' vascular system (Toth et al., 2011; Czajkowski et al., 2012).

3.1 The enemy - Soft Rot Enterobacteriaceae

Bacteria from genera *Dickeya* and *Pectobacterium* form a group called Soft Rot Enterobacteriaceae or Soft Rot Erwiniae (SRE). They all belong to family Enterobacteriaceae. The SRE are found worldwide and have been isolated from plants of more than half of angiosperm families as well as from soil, rivers, ground water, insects, mollusks and

nematodes (Perombelon and Kelman 1980; Pérombelon and Salmond 1995; Ma et al., 2007, Laurila et al., 2008; Nykyri et al., 2014; Parkinson et al., 2014). They are Gram negative, rod-shaped, facultative anaerobes that produce a wide range of plant cell-wall degrading enzymes (PCWDE) and cause diseases such as blackleg and soft rot. They possess a wide range of hosts including 16 dicotyledonous families of plants in 11 orders and 10 monocotyledonous families in 5 orders (Samson et al., 2005; Ma et al., 2007). Soft rot losses may occur in the field, garden, greenhouse, or after harvest during transit, storage, or marketing. The SRE have a climatic distribution that reflects their host diversity and growing temperatures. *Pectobacterium atrosepticum* - Pba (formerly *Erwinia carotovora* subsp. *atrosepticum*, Eca) is mainly restricted to temperate climates and almost exlusively to potatoes. *Pectobacterium carotovorum* subsp. *carotovorum* - Pcc (formerly *Erwinia carotovora* subsp. *carotovora*, Ecc) infects a wide diversity of plants and is found in both temperate and tropical zones, causing soft rot to potatoes and many fruits and vegetables (Elphinstone 1987; Toth 2003). *Dickeya* spp. (formerly *Erwinia chrysanthemi*, Ech) affect a wide diversity of tropical and subtropical plants, including potatoes, many ornamental plants, maize, rice and pineapple. *D. dianthicola* causes disease of many ornamental plants worldwide, but also has played an important role in potato losses in Europe since 1970s (Toth et al., 2011). In Table 1 the exemplary hosts of different SRE have been presented.

Table 1. Characteristics of the *Dickeya* spp. and *Pectobacterium spp.* host species

Species name	Host range	References
Dickeya aquatica	Isolated from water, host plant not determined	Parkinson et al. 2014
Dickeya chrysanthemi bv. chrysanthemi	*Chrysanthemum spp., Cynara scolymus, Cichorium intybus, Helianthus annuus*	Samson et al., 2005
Dickeya chrysanthemi bv. parthenii	*Lycopersicon esculentum, Parthenium argentatum Philodendron spp.*	Samson et al., 2005
Dickeya dianthicola	*Dianthus spp., Cichorium intybus, Cynara scolymus, Dahlia variabilis, Kalanchoe blossfeldiana, Lycopersicon esculentum.* ***Solanum tuberosum***.	Samson et al., 2005

Dickeya dadantii subsp. *dadantii*	*Pelargonium capitatum*, *Ananas comosus*, *Dianthus spp.*, *Euphorbia pulcherrima*, *Ipomoea batatas*, *Musa spp.*, *Philodendron spp.*, *Saintpaulia ionantha*, *Zea mays*	Samson et al., 2005
Dickeya dadantii subsp. *dieffenbachiae*	*Dieffenbachia spp.*, *Lycopersicon esculentum*, *Musa spp.*	Brady et al., 2012
Dickeya paradisiaca	*Musa spp.*, *Zea mays*	Samson et al., 2005
Dickeya solani	***Solanum tuberosum***	Van der Wolf et al., 2014
Dickeya zeae	*Zea mays*, *Ananas comosus*, *Brachiaria ruziziensis*, *Chrysanthemum morifolium*, *Musa spp.*, *Nicotiana tabacum*, *Oryza sativa*, ***Solanum tuberosum***,	Samson et al., 2005
Pectobacterium atrosepticum	***Solanum tuberosum***, *Solanum lycopersicum*, *Cichorium intybus*	Gardan et al., 2003
Pectobacterium betavascularum	*Beta vulgaris*	Gardan et al., 2003
Pectobacterium carotovorum subsp. *carotovorum*	***Solanum tuberosum***, *Beta vulgaris*	Hauben et al., 1998
Pectobacterium carotovorum subsp. *brasiliense*	***Solanum tuberosum***	Hauben et al., 1998
Pectobacterium carotovorum subsp. *odoriferum*	***Solanum tuberosum***, *Cichorium intybus*	Hauben et al., 1998
Pectobacterium wasabiae	*Armoracia rusticana*, ***Solanum tuberosum***	Gardan et al., 2003

The most recent taxonomy of SRE states that those bacteria belong to the kingdom of Bacteria, phylum Proteobacteria, class Gammaproteobacteria, order Enterobacteriales, family Enterobacteriaceae. Since 1998 SRE have been divided in two genera: *Pectobacterium* and *Dickeya* (Hauben et al.).

The taxonomy of SRE has been changing over the past decades. For the first time the genus *Erwinia* was described in 1917 - it comprised all members of the Enterobacteriaceae that were pathogenic to plants, including both pectinolytic (e.g. *Erwinia carotovora* and *Erwinia chrysanthemi*) and non-pectinolytic (*Erwinia amylovora*) species (Winslow et al., 1917). The name was chosen after of one of the first phytopathologists – Erwin Smith. In 1953, Burkholder and colleagues assigned *E. chrysanthemi* to the genus as a pathogen of chrysanthemum. Later studies proved that *E. chrysanthemi* cause disease on a wide variety of plant hosts (Samson et al., 2005; Ma et al., 2007; Table 1). In 1984, Lelliott and Dickey, on the basis of host specificity subdivided the species *E. chrysanthemi* into six pathovars, namely pvs *chrysanthemi, dianthicola, dieffenbachia, paradisiaca, parthenii* and *zeae*. In 1987, Samson and colleagues developed a biovar system based on some biochemical characteristics.

The moving of pectinolytic erwiniae into a new genus *Pectobacterium* based on their ability to produce pectinolytic enzymes was already proposed by Waldee in 1945, but it was not done until 1998 when 16S rDNA analysis provided more data for this proposal (Hauben et al., 1998). Whilst the potato pathogens *P.c.* subsp. *carotovorum* (syn. *E c.*subsp. *carotovora*) and *P. atrosepticum* (syn. *E.c.* subsp. *atroseptica*) remain within this genus, further analysis of *P. chrysanthemi* using 16S rDNA, DNA–DNA hybridization and biochemical characterization showed that it forms a distinct clade from *Pectobacterium* spp., so a new genus, *Dickeya*, was proposed (named after the microbiologist Robert S. Dickey) (Samson et al., 2005). Then, Brady and colleagues (2012) reclassified *D. dieffenbachiae* into *D. dadantii* subsp. *dieffenbachiae*. In 2014 two new species of *Dickeya* were proposed: *D. aquatica* and *D. solani* (Parkinson et al., 2014, Van der Wolf et al., 2014). All currently accepted species and subspecies of SRE are presented in Table 1.

3.2 Soft rot and Blackleg symptoms – what are we dealing with?

SRE cause blackleg and soft rot diseases. They are often present in latent infections on many host crops (Perombelon and Kelman, 1980). The symptoms of soft rot are similar on most plants. The disease starts on leaves, stems, and/or underground parts as small, water-soaked, translucent lesions which rapidly enlarge in both diameter and depth. The host tissue softens and becomes squashy or watery. Slimy masses of bacteria and cellular debris frequently seep out from cracks in the tissues. In optimal conditions, within 20 to 72 hours,

entire fleshy fruits, roots, tubers, stems and rhizomes, bulbs, corms, buds, leaf stalks, and leaves may rot and collapse, sometimes leaving only the outer “skin” intact. The decaying tissue, may be opaque, white, cream-colored, gray, brown, or black and is always wet. Sometimes characteristic putrid odor occurs, caused by secondary invading bacteria that are growing in the decomposing tissues (Agrios 2005).

Infected potato tubers may rot in wet soil, so then no new plants apear. Shoots arising from infected tubers become watery, wilt, and then collapse. This potato disease is commonly called blackleg. Blackleg is soft rot that spreads from infected seed tubers into the stems of new potato plants (it is a seed-borne disease). If the soil moisture level is lowered, the base of the shoots may become soft, brown to inky-black, and shriveled. The leaves on such shoots are dwarfed, stiff, curled upward, and are yellowish, red, or bronzed. Affected shoots are also stunted, more upright, and pale in color. Such plants often die prematurely or their yield is reduced (RPD No. 943 July 1990). In Figure 1 are presented examples of soft rot on different hosts and blackleg of potato.

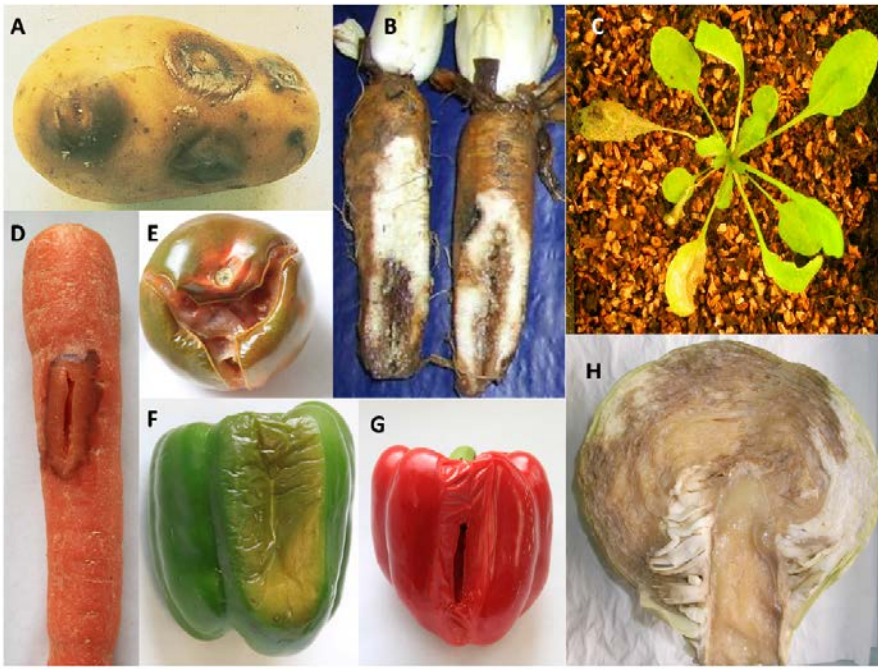

Figure 1. Symptoms of soft rot on different hosts and example of blackleg on potato. A. Potato tuber. B. Chicory roots. C. Arabidopsis thaliana plant. D. Carrot root. E. Tomato fruit. F and G. Green and red pepper. H. Cabbage. Photo from Reverchon and Nasser 2013. I. Potato plant: i. Leaf wilting. ii. Blackleg. J. Soft rot of potato section. Photos made by M. Potrykus.

3.2.1 SRE disease cycle

For disease development the compatibile interaction has to be established between the bacterial strain and the host plant. In addition the environmental conditions have to be optimal for disease development. There is not only plant and pathogen interaction, but also an influence of the environment. Under favorable conditions (i.e. for SRE high humidity and optimal temperature) the disease can develop.

SRE can live as epiphytes or as saprophytes in soil on the plant debris until they encounter a susceptible host. They are strong competitive bacteria that exclude other microbes by contact-dependent growth inhibition mechanisms involving type V and type VI secretion systems (Aoki et al., 2010; Koskiniemi et al., 2013, Pedron et. al 2014). SRE can also infect insects, which may then serve as dissemination vectors (Grenier et al., 2006). Nykyri and colleagues (2014) also showed that nematodes can serve as a vector spreading SRE. The three main steps of plant infection by SRE are: 1) adhesion to the plant surface and penetration in the plant tissues, either *via* wounded sites or through natural openings such as stomata,

2) invasion of the apoplasts and 3) plant cell wall degradation. SRE can reside in the vegetal intercellular spaces, causing a latent infection without any symptoms (Lebeau et al., 2008). If the bacteria encounter advantageous conditions, such as favorable temperature, high humidity levels and poor oxygen availability, then a shift to disease occurs (Pérombelon and Kelman, 1980; Pérombelon and Salmond, 1995). Each of these disease stages requires the detection of information from the environment, the host and the pathogen population. In the minireview of Reverchon and Nasser (2013) there is presented a list of genes involved in disease development that have been studied mostly in *D. dadantii* 3937strain (Table 2). This strain has been isolated from *Saitpaulia ionantha* (Kotoujanski et al., 1982) and most of the genetic studies and virulence factors analyses have been performed on this strain, therefore it has been accepted by a scientific community as a model for molecular study of *Dickeya* spp.

Dickeya spp. disease cycle on potatoes is presented in Figure 2. During the spring, *Dickeya* spp. are commonly found on or in the seed tubers, which represent the primary inoculum. In the late spring and in summer, bacteria from infected seed spread into young stems and roots. Bacteria multiply in intercellular spaces, degrade the plant cell walls and liquefy the mother tuber. Progeny tubers may become contaminated with *Dickeya* spp. as they develop in the late summer and early fall. Emerging bacteria may migrate through the soil in water and infect the neighbouring plants. Soft rot occurs when the bacteria gain access to the tuber through wounds and other entry points. Wounds caused during harvest and handling provide multiple points of entry into the tuber, so the infection may spread within the storage containers during storage. Discarded rotten tubers may allow dispersal of *Dickeya* spp. in environment (in soil and water). Bacteria from different species of *Dickeya* and *Pectobacterium* are able to grow and produce virulence factors in different temperatures. For example, Perombelon and colleagues (1987) showed that soil temperature of 20 ºC was an important transition point, above which *E. carotovora* subsp. *atroseptica* (currently *P. atrosepticum*) and below which *E. chrysanthemi* (currently *Dickeya* spp.) were not apparently pathogenic. During winter, *Dickeya* spp. can survive in association with plant residues in the soil. During the whole disease cycle, insects could be involved in disease development either because they cause wounds on plant organs on which they feed or deposit their eggs and these allow bacteria to enter the plant, or they transfer bacteria to other plants where they might cause disease (Reverchon and Nasser 2013).

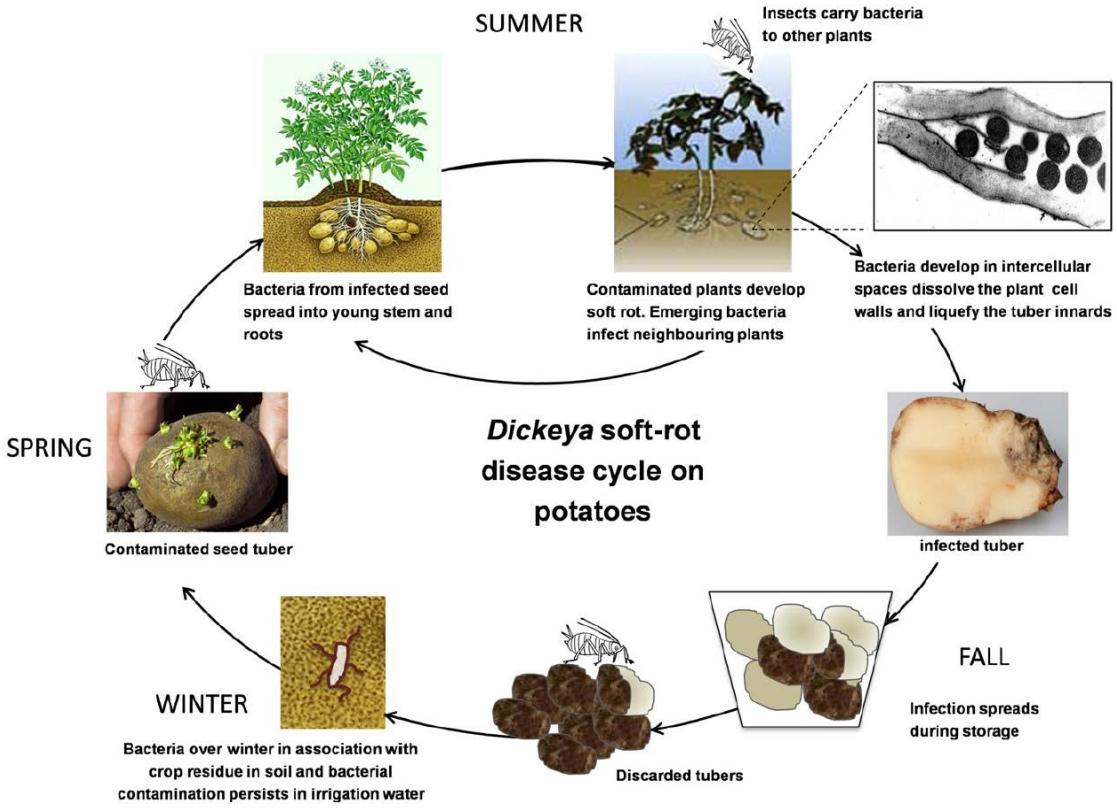

Figure 2. *Dickeya* spp. disease cycle. Reverchon and Nasser 2013.

Recently, Reverchon and Nasser (2013) described a wide range of genes that may play a crucial role in different stages of plant-microbe interactions. Genes encoding proteins playing important roles in epiphytic interaction, early, intermediate and late stage of infection are listed in Table 2.

Table 2. Genes involved in *Dickeya* spp. disease development. After Reverchon and Nasser 2013.

Gene name	Stage of infection	Description
bcsABCD	Epiphytic	Cellulose microfibrils synthesis, adhesion to plant surface
wza-wzb-wzc	Epiphytic	Exopolysaccharide synthesis, adhesion to plant surface
hecBA2/cdiBA2	Epiphytic	Type V autotransporter contact-dependent inhibition system contributing to bacterial adhesion to plantsurface
rhsA	Epiphytic	Intercellular competition system
rhsB	Epiphytic	Intercellular competition system
rhsC	Epiphytic	Intercellular competition system
impBCGHJKAL	Epiphytic	Type VI secretion system involved in Rhs secretion
rhlA	Epiphytic	Biosurfactant synthesis, colonization of plant surface
motABcheAWDRBYZ	Epiphytic and other	Flagella motor and chemotaxis, entry into plant apoplast

cfa	Early	Cyclopropane fatty acid synthesis, resistance to acidic pH
asr	Early	Acid shock periplasmic protein, resistance to acidic pH
arnB-T	Early	Enzymes involved in LPS modification, resistance to cationic antimicrobial peptides
sapABCDF	Intermediate	Antimicrobial peptide import system, resistance to antimicrobial peptides
ohr	Intermediate	Thiol peroxidase, resistance to lipid hydroperoxide
sodA	Intermediate	Superoxide dismutase, resistance to oxidative stress
sodC	Intermediate	Superoxide dismutase, resistance to oxidative stress
katG	Intermediate	Catalase-peroxidase, resistance to oxidative stress
katE	Intermediate	Catalase-peroxidase, resistance to oxidative stress
ahpCF	Intermediate	Alkyl hydroperoxide reductase, resistance to oxidative stress
indABC	Intermediate	Indigoidine biosynthesis, resistance to oxidative stress
msrA	Intermediate	Methionine sulfoxide reductase, resistance to oxidative stress
acsF-A	Intermediate	Achromobactin synthesis, iron scavenging
cbrABCD	Intermediate	Achromobactin transport system, iron scavenging
fct-cbsCEBAP	Intermediate	Chrysobactin synthesis and receptor, iron scavenging
ftnA	Intermediate	Maxi-ferritin, iron storage, iron homeostasis
bfr	Intermediate	Maxi-ferritin, iron storage, iron homeostasis
dps	Intermediate	Mini-ferritin, iron storage, iron homeostasis
sufABCDSE	Intermediate	Iron-sulphur cluster assembly system
iscRSUA	Intermediate	Iron-sulphur cluster assembly system
hrpA-hrpE	Intermediate	Type III secretion system
hrpN	Intermediate	Harpin secreted by type III system
hrpW	Intermediate	Harpin secreted by type III system
dspEF	Intermediate	Type III effectors
budAB	Intermediate	Butanediol fermentative pathway
iaaMH	Intermediate Late	Auxin production
pelA-pelE-pelD	Late	Pectate lyases involved in pectin degradation
pelB-pelC	Late	Pectate lyases involved in pectin degradation
pelL	Late	Pectate lyases involved in pectin degradation
ganEFGABC	Late	Degradation of galactan, a major component of the ramified regions of pectin
celZ	Late	Cellulase involved in cellulose degradation
outC-outO	Late	Type II secretion system required for pectate lyases and cellulase secretion
prtG-inhprtDEFBCA	Late	Proteases G, A, B, C and their type I secretionsystem PrtDEF

3.3 Armory – virulence factors of SRE

SRE use a combination of different compounds, such as plant hormons (auxin), pectin metabolites, acyl-homoserine lactones, and organic acids that regulate expression of genes encoding pectate lyases and other virulence factors at both transcriptional and posttranscriptional stages and it is possible to interfere with soft-rot pathogenicity by disrupting these signaling cascades (Charkowski, 2009, 2012). The main weapon in the SRE arsenal is the coordinated production of high levels of multiple exoenzymes, including pectinases, cellulases and proteases, which break down plant cell walls and release nutrients for bacterial growth (Barras et al., 1994; Py et al., 1998; Thomson et al., 1999; Pérombelon, 2002; Hugouvieux-Cotte-Pattat et al., 1996 and 2014).

3.3.1 Plant cell wall degrading enzymes (PCWDE)

3.3.1.1 Pectinases produced by Dickeya spp.

The activity and regulation of pectinases produced by *D. dadantii* 3937 was described by Hugovieux-Cotte-Pattat and colleagues in 1996 and reviewed in 2014. The term 'pectinases' describes different enzymes cleaving the glycosidic linkages or the methyl-ester bonds of the pectic polymers. Many of these pectinases - pectate lyase (Pel), pectin lyase (Pnl), pectin methyl esterase (Pme) and polygalacturonase (Peh) exist in multiple forms (isoenzymes) encoded by independent genes that, in some cases at least, are clustered and appear to be derived from successive rounds of gene duplication (Barras et al., 1987; McMillan et al., 1994). The *D. dadantii* 3937 strain produces two pectin methylesterases, at least nine pectate lyases, a polygalacturonase, and a pectin lyase.

Historically, pectate lyases isolated from various organisms were often named as PelA, B, C or Pel-1, 2, 3, *et cetera*, depending on either their order of discovery or their characteristics (i. e. their isoelectric point in the case of the first enzymes described in *D. dadantii* 3937, Bertheau et al., 1984). With such a nomenclature, orthologous pectate lyases often have different names in various strains or SRE species. Pectate lyases (Pels) are the main pectinases in pathogenesis and, as with other exoenzymes, their number varies between species, subspecies and strains. Pectate lyases generally have basic pH (from 7.3 to 9.5) optima and an absolute requirement for a divalent cation (Ca^{2+} in most cases). They preferentially cleave polygalacturonate (PG) or partially methylated PG. However, the

enzymatic properties can differ significantly among the different pectate lyases. For instance, the optimal PG methylation level varies from 0 to 50 % among the *D. dadantii*3937 pectate lyases (Table 3). There are generally five major Pels which are grouped in two families (Pel A, D, E and Pel B, C) and at least four secondary Pels (Pel I, L, Z and X) in *D. dadanti* 3937. The secondary Pels have a lower enzymatic activity than the major ones, but appear to play an important role in either the early phase of infection or host specificity (Lojkowska et al., 1995; Pissavin et al., 1996; Shevchik et al., 1997; Jafra et al., 1999). Additional isoenzymes of Pel, Pnl, Pme and Peh are also induced in minimal medium in the presence of both pectate and pectin (McMillan et al., 1994). Pectinases are secreted through the commonly named Out secretion system (Type II Secretion System, T2SS). In Figure 3 the scheme of pectinolytic enzymes actions is presented. For better understanding the scheme in Figure 3, the original explanation from Hugouvieux-Cotte-Pattat and colleagues 2014) is shown under the figure.

Table 3. Characteristics of *D. dadantii* 3937 pectate lyases and related proteins. After Hugouvieux-Cotte-Pattat et al., 2014

Name	PL family (subfamily)	Substrate (optimal methylation)	Optimal pH	Cation(s)	Cell location (secretion system)
PelZ	1 (2)	PG (0 to 20%)	8.5-9.0	Mn (Ca)	external (Out)
PelB	1 (3)	PG (0 to 40%)	9.0-9.5	Ca	external (Out)
PelC	1 (3)	PG (0 to 20%)	9.0-9.5	Ca	external (Out)
PelA	1 (6)	PG (0%)	8.5	Ca	external (Out)
PelD	1 (6)	PG (0%)	8.5	Ca	external (Out)
PelE	1 (6)	PG (0%)	8.0	Ca	external (Out)
PnlH	1 (8)				OM (Stt)
PelW	2 (2)	G3, G4, PG (0 to 60%)	8.5	Co, Mn, Ni	cytoplasm
HrpW [a]	3 (1)				external (Hrp)
PelI	3 (5)	PG (0 to 50%)	9.2	Ca	external (Out)
RhiE	4 (4)	RGI	6.2	no	external (Out)
PelL	9 (1)	PG (0 to 30%)	8.0-9.0	Ca	external (Out)
PelN	9 (1)	PG (0 to 90 %)	7.4	Fe	external (Out)
PelX	9 (1)	G4 to G7, PG (0 to 20%)	8.0-8.5	Ca (Mn, Co, Ni)	periplasm
OGL	22	G2, G3, uG2, uG3	7.3-7.7	Mn, Co, Ni, Fe	cytoplasm

a - This protein possesses a PL3 domain that binds to PG, but has no enzymatic activity.
Abbreviations: PG, polygalacturonate; G2, G3 etc, saturated di-, tri- galacturonates; uG2, uG3 etc, unsaturated di-, tri- galacturonates; DKI, 4-deoxy-L-*threo*-5-hexosuloseuronic acid (or 5-keto-4-deoxyuronate); OM, outer membrane, Out – type 2 secretion system, Hrp – type 3 secretion system.

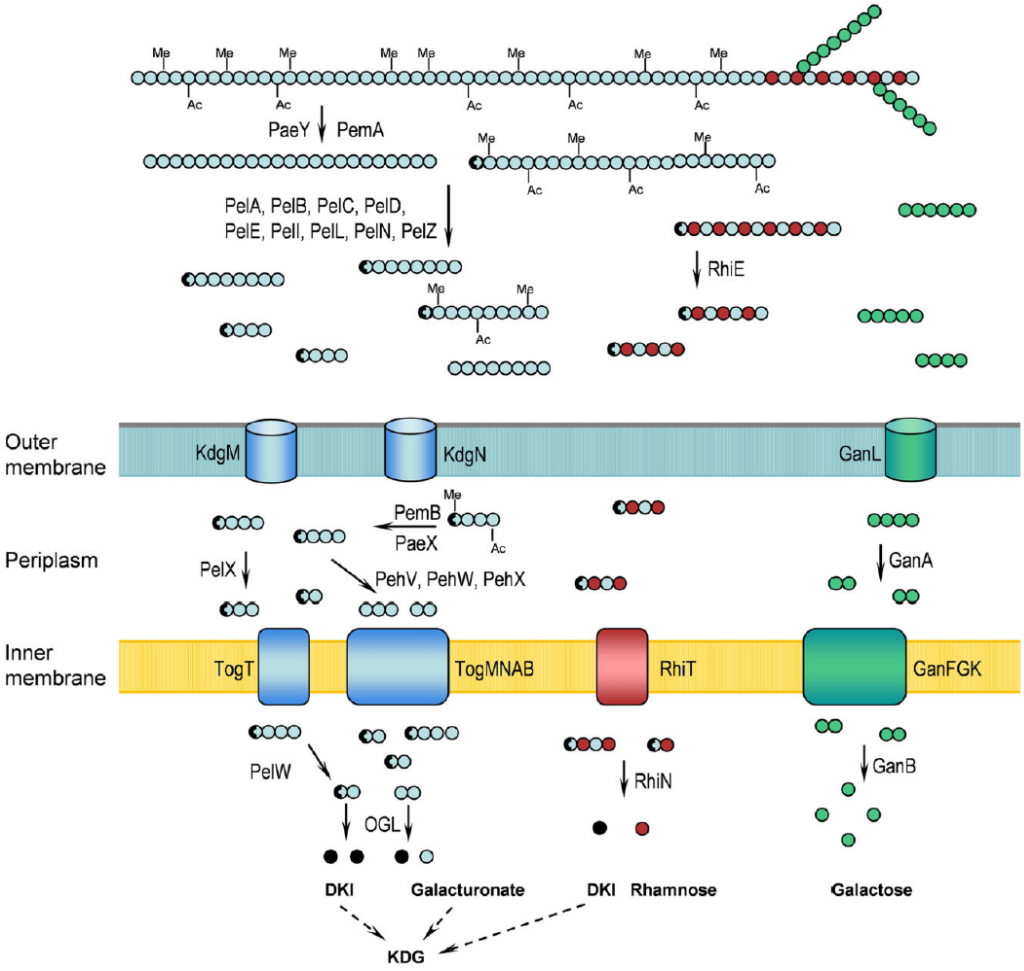

Figure 3. Scheme of pectinolytic enzymes action and presence in the bacterial cell. Pectin degradation pathways in *Dickeya dadantii*. Methyl-esterification and acetylation of the pectin linear regions are removed by extracellular esterases, PemA and PaeY respectively. The PG backbone is cleaved by the secreted endo-pectate lyases PelA, PelB, PelC, PelD, PelE, PelI, PelL, PelN and PelZ. The resulting unsaturated oligogalacturonates enter the periplasm through the porins KdgM and KdgN. In the periplasm, the remaining methyl and acetyl groups are removed by PemB and PaeX respectively, and oligomers are cleaved by the exo-pectate lyase PelX and the exo-polygalacturonases PehV, PehW and PehX. The resulting small oligomers enter the cytoplasm using the transporters TogT and TogMNAB. Finally, the cytoplasmic enzymes PelW and OGL generate two monomers, 5-keto-4-deoxyuronate (DKI) and galacturonate, which are metabolized in the cytoplasm into 2-keto-3-deoxygluconate (KDG). The rhamnogalacturonan I (RGI) backbone, a chain of alternating L-rhamnose and D-galacturonic acid residues, is cleaved by the rhamnogalacturonate lyase RhiE. The porin, allowing the resulting oligomers to enter the periplasm, has not been identified. These oligomers use the transporter RhiT to enter the cytoplasm, whereas the enzyme RhiN cleaves the terminal unsaturated galacturonate residue. The galactan side chains of RGI are also metabolized. Oligogalactans enter the periplasm through the GanL porin. The periplasmic endo-galactanase GanA generates short oligomers that use the GanFGK transport system to cross the inner membrane. Finally, the cytoplasmic exo-galactanase GanB cleaves oligogalactans into galactose. (Hugouvieux-Cotte-Pattat et al. 2014).

3.3.1.2 Cellulolytic enzymes produced by SRE

The complete degradation of native cellulose to glucose requires three enzymes: *endo-β-I*,4-glucanase, cellobiohydrolase and β-glucosidase. Cellulases, which exhibit mainly endoglucanase (EG) activity, break down cellulose in the primary and secondary cell walls of the host plant. *D. dadantii* 3937 produces two EGs: EGZ, which comprises the major activity and is secreted (Boyer et al. 1984), and EGY, which is less abundant and is periplasmic (Giuseppi et al., 1991). The genes encoding these enzymes are *celZ* and *celY*, respectively. They seem to be inessential for pathogenicity, but they do appear to act in synergy with other exoenzymes of various classes (i e. pectinases) to attack the plant cell wall (Boccara et al., 1994). EGZ is secreted by the so-called Out general secretion pathway (T2SS). EGZ contains two functionally independent domains: a catalytic domain (CD) and a cellulose-binding domain (CBD) joined by a Ser/Thr-rich linker region (LR) (Py et al., 1991).

3.3.1.3 Proteases produced by SRE

Many plant-pathogenic bacteria produce proteases. *E.chrysanthemi* strain produces metalloproteases A, B, C and G (Delepelaire and Wandersman, 1990; Ghigo and Wandersman, 1992). The proteases are secreted into the external medium via Type I Secretion system, two of them as inactive precursors (zymogens B and C) where they are activated by divalent cations. Metalloproteases may play a role in virulence by degrading plant cell wall proteins. They may act either to provide amino acids for biosynthesis of microbial proteins or degradation of host proteins associated with resistance (Heilbronn and Lyon, 1990; Kyöstiö et al., 1991), but according to the literature, like cellulases, they appear to play only a minor role in pathogenesis (Marits et al., 1999).

3.3.2 Motility

Phyto-pathogenic bacteria can be motile by means of flagella and genes coding for flagellar proteins contribute to virulence and to host-pathogen interactions, i.e. for SRE – potato interaction (Van Vaerenbergh et al. 2012). Two different types of motility require the presence of bacterial flagella: ‘swimming’, which takes place in liquid media, and ‘swarming’ motility, on solid surfaces or in media of high viscosity (Harshey, 2003). The ability to swarm can be dependent upon both growth media and temperature (Jahn et al., 2008). The analysis of the virulence of the mutants in different host plants indicated that motility and chemotaxis play an important role in the pathogenicity of *D. dadantii* 3937 (Antunez-Lamas et al.,

2009ab). In plant pathogenic bacteria, the involvement of motility in power pathogen has been relatively well studied in *Agrobacterium tumefaciens* in interactions with plants. This bacterium produces flagella disposed circularly at one end of the bacillus-shaped cell. Three genes, *flaA, flaB* and *flaC* are involved in the synthesis of flagella and in determining the pathogenicity of this bacterium (Chesnokova et al., 1997). In the case of plant pathogenic bacterium *P. carotovorum*, non-mobile mutants (not possessing flagella) exhibit attenuated virulence (Mulholand et al., 1993; Pirhonen et al., 1991). Non-chemotactic but motile mutants of *Ralstonia solanacearum*, an economically important pathogen of *Solanaceous* plants, showed significantly reduced virulence with respect to the wild-type in the host plant tomato, demonstrating that directed motility, not simple random motion, is required for full virulence (Yao & Allen, 2006). The results presented by Antunez-Lamas and colleagues (2009a) indicated that mutations affecting motility/chemotaxis (in genes *motA* and *cheY*) had an influence on the *D. dadantii* 3937 virulence toward several hosts: chicory, *Saintpaulia* and potato. Mutants *motA* and *cheY* showed a significant reduction of their virulence in those hosts. Motility/chemotaxis also plays a role in colonization of potato tubers (Antunez-Lamas et al., 2009b).

3.3.3 Iron uptake

Another process that is crucial for pathogenesis is iron uptake, which was firstly linked to pathogenicity in *Dickeya dadantii* 3937 through the analysis of bacteriocin-resistant mutants (Expert and Toussaint, 1985). Iron is a necessary cofactor for enzymes involved in important cellular functions. SRE regulate genes expression in response to iron, for example low iron availability is a signal that triggers transcription of the genes encoding major pectate lyases PelD and PelE as well as that of genes involved in iron transport. This regulation is mediated by the transcriptional repressor Fur (Franza et al., 1999 and 2002). *D. dadantii* 3937 produces the siderophores chrysobactin and achromobactin in order to acquire iron from the iron-poor environment of the plant apoplast. Several studies demonstrated that the presence of chrysobactin and achromobactin highly contributes to the successful infection of the plant (Enard et al. 1988; Franza et al. 2005; Dellagi et al. 2005). Mutants defective in chrysobactin-mediated iron transport remain localized within *Saintpaulia* leaves, suggesting a role in bacterial spread throughout the plant (Enard et al., 1988). Achromobactin deficient mutants are also affected in their virulence, but are more aggressive than the chrysobactin non-

producers and double mutants deficient in both achromobactin and chrysobactin production, are impaired in symptom initiation.

An analysis of *Dickeya* spp. and *Pectobacterium* spp. genomes revealed multiple TonB-dependent outer membrane receptors and TonB homologs (TonB-dependent transporters are bacterial outer membrane proteins that bind and transport ferric chelates), suggesting that the capacity of using diverse exogenous siderophores is common among SRE and may confer fitness in complex environments (Schauer et al., 2008). A study of *P. atrosepticum* SCRI1043 and *D. dadantii* 3937 revealed that besides the production and utilization of siderophores, they have the capacity to use other iron sources through siderophore-independent systems, such as Feo system, Efe UOB system or Hmu system (reviewed by Franza and Expert 2013). Both species are able also to uptake the haem iron, while *P. atrosepticum* SCRI1043 only can transport the ferric citrate complex and only *D.dadantii* 3937 can aquire ferrous iron system (Franza and Expert 2010).

3.3.4 *Quorum sensing* communications systems

Virulence genes are expressed in a concerted manner and culminate when bacterial quorum is reached. Numerous Gram-negative species use AHL-based quorum sensing to regulate expression of genes involved in interactions with host cells. In *Pectobacterium* spp. which produces 3-oxo-C6-HSL and 3-oxo-C8-HSL, the AHL quorum-sensing system is at the top of a regulatory cascade controlling over a quarter of the *Pectobacterium* spp. genes, including key virulence factors, such as pectinase, cellulase, and protease activities, and production of HrpN, a protein secreted via type III secretion system (T3SS) (Liu et al., 2008; Charkowski et al. 2012). Expression of the virulence genes encoding plant cell wall-degrading enzymes is only activated when a sufficient amount of the AHL signal molecule has accumulated (Andersson et al., 2000). Expression of those genes is also induced by pectin metabolites. Pectin is both one of the main components of the plant cell wall and a carbon source that soft rot pathogens are able to exploit. In contrast, in the closely related *D. dadantii* 3937, AHL appear to play a minor role in virulence gene regulation. Specific interactions have been observed in vitro between the AHL-related quorum sensing regulator ExpR and *pel* gene promoters, but no specific phenotype was associated to the *expI* or *expR* mutations (Nasser et al., 1998). In addition to ExpI-ExpR, the LuxS-based quorum sensing system of *D.*

dadantii 3937 does not play a pivotal role in the cell density-dependent control of virulence gene expression *in vitro* or *in planta* (Reverchon et al., 1998; Mhedbi-Hajri et al., 2011).

Besides AHL-based quorum sensing, a new communication system has beed discovered in *D. dadantii* 3937 called Virulence Factor Modulating cluster (Vfm) (Nasser et al., 2013). The Vfm cluster does not seem to be widespread among bacterial species, but is conserved in *Dickeya* species, the Vfm cluster is absent from the related pectinolytic enterobacteria of the *Pectobacterium* genus (Nasser et al., 2013). Studies on the mutants with decreased production of all PCWDE that could be complemented by cell-free culture supernatant have revealed that Vfm cluster controls the transcription of the PCWDE genes (Nasser et al., 2013).

3.4 Not only the bullets, also the guns. Secretion systems and their role in SRE cells

Protein export is the key in the SRE virulence and in their genomes all six known secretion systems in Gram-negative bacteria are present (Glasner et al., 2011; Garlant et al., 2013; Pedron et al., 2014). These systems are involved in attacking host plants and competing bacteria (Charkowski et al., 2012).

The rapid induction of expression of genes coding for exoenzymes and other pathogenicity factors within the bacterial cell is of little consequence unless they can be efficiently targeted to the extracellular environment. To accomplish this, SRE have all six secretion systems known for Gram-negative bacteria, all of which exhibit very different mechanisms that appear to be conserved between different bacterial species both within and outside the *Dickeya* and *Pectobacterium* genera. Figure 4 presents the schematic composition of each secretion systems.

Type I Secretion System (T1SS) secretes proteases from the cytoplasm to the extracellular space in a single step, but, while this system has been studied in detail in *Dickeya* spp., it appears to have a relatively minor role in pathogenicity (Dahler et al., 1990; Delepelaire and Wandersman, 1990; Létoffé et al., 1990). In *Pectobacterium* spp., the T1SS is upregulated by plant extracts and acyl-homoserine lactone (AHL), and controlled by GacAS (Marits et al., 1999 and 2002). In *Dickeya* spp., this system is controlled by PecS (Hommais et al., 2008; Mhedbi-Hajri et al., 2011) and GacAS (Lebeau et al., 2008). T1SS-associated ABC transporters with a noncatalytic C39 peptidase-like domain tend to secrete large, 50 ~1,000

kDa, repeats-in-toxin (RTX) proteins with uncleaved C-terminal secretion signals (Linhartova et al. 2010). In *D. dadantii* 3937, RTX proteases (PrtG, PrtB, PrtC, PrtA) are secreted by a T1SS (Letoffe et al., 1989; Delapelaire and Wandersman 1991; Ghigo and Wandersman, 1992; Kazemi-Pour et al., 2004). Two of the proteases (PrtB, PrtC) are secreted as zymogens that are activated after secretion by divalent cations (Delapelaire and Wandersman 1989). This characteristic is similar to other RTX metalloproteases, which belong to a subgroup of proteases with an extended zinc-binding motif (Linhartov´a et al. 2010). *D. dadantii* 3937 also exports a protease inhibitor to the periplasmic space in a Sec-dependent manner that is superficially analogous to the contact-dependent growth inhibition mechanism, but the inhibitor is non-specific and can inhibit many proteases (Letoffe et al., 1989). Additionally, RTX protease mutants of *P. carotovorum* and *E. amylovora* are modestly affected in virulence, suggesting a role in plant pathogenesis rather than toxicity against other bacteria (Marits et al., 1999; Zhang et al., 1999).

T2SS (also called Out system), on the other hand, is essential for pathogenicity and secretes pathogenicity determinants such as pectinases and cellulases in a two-step mechanism. The first step is a Sec-dependent protein export system that exports proteins to the periplasm. The second step, controlled by a 15-gene out cluster, includes the formation of a structure that spans the periplasmic compartment and outer membrane and channels proteins, recognized by a signal sequence, to the outside of the cell. Type II–secreted proteins have been identified during individual enzyme studies and enzyme or global secretome analyses of *D. dadantii* or *P. atrosepticum* (Salmond et al. 1994; Coulthurst et al., 2008., Kazemi-Pour et al., 2004). However, despite a high level of interspecies amino acid identity within the SRE, out genes from *P. c*. subsp. *carotovorum* (strain SCRI193) do not complement mutations in equivalent genes in *D. dadantii* 3937 and vice versa, suggesting a degree of species-specificity (Py et al., 1991). Regulation of Type II system is, at least in part, under the control of KdgR and may also operate under a quorum-sensing mechanism (Condemine and Robert-Baudouy, 1995; Condemine et al., 1992).

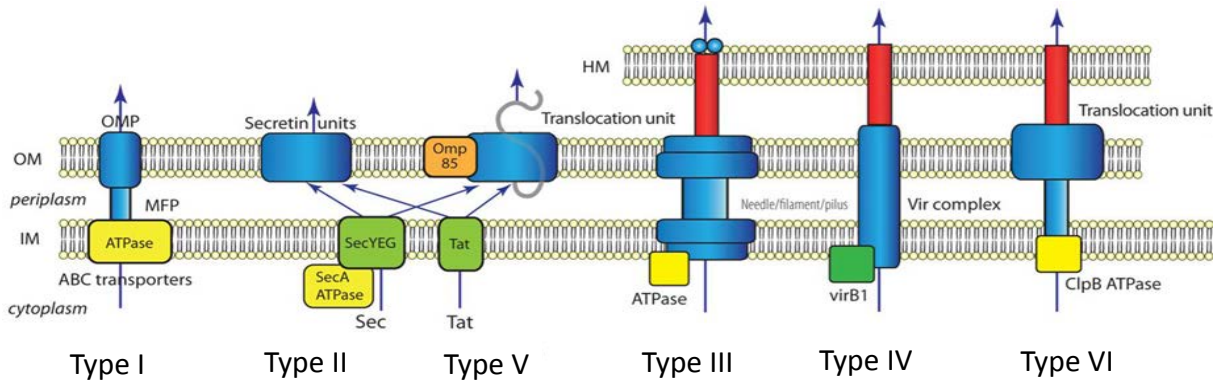

Figure 4. Schemes of secretion systems present in SRE. After Tseng et al. 2009. HM- host membrane, OM – outer membrane, IM – inner membrane.

Type III secretion system (T3SS) has been more closely examined in hemibiotrophic phytopathogenic bacteria, such as *Pseudomonas syringae*, than in SRE, but is required for patho- genesis in both bacterial groups. Unlike *P. syringae,* which can have up to 30 potential type III–secreted effector proteins in individual strains, SRE appear to have relatively few (Holeva et al., 2004; Kim et al., 2011), including a small number of harpins or helper proteins and the single known effector, DspA/E. Holeva and colleagues (2004) demonstrated that the T3SS helper HrpN and effector DspE/A are required for pathogenicity in *P. atrosepticum*.

In comparison to other secretion systems, type IV secretion system (T4SS) is unique in its ability to transport nucleic acids in addition to proteins into plant and animal cells, as well as into yeast and other bacteria (Christie et al., 2005). Genes that encode a similar system were discovered in the complete genome sequence of *P. atrosepticum* SCRI1043, and further investigation revealed that a mutation in the T4SS reduced its virulence on potato (Bell et al. 2004).

Type V secretion systems (T5SSs) have relatively simple structures and they are reviewed by Leo and colleagues (2012). T5SSs are subtyped as T5aSS–T5eSS on the basis of differences in the structure and mechanism of secretion. The functions of the T5SS secreted proteins are variable, but include serine proteases, lipases, cytotoxins, invasins, and adhesins that collectively generalize to influencing bacterial fitness, aggregation, biofilm formation, and virulence (Grijpstra et al., 2013). The T5SSs have also been implicated in a phenomenon referred to as contact-dependent growth inhibition, where, upon contact, toxic proteins are targeted to another bacterium (Ruhe et al., 2013). In *D. dadantii*, a >3,800 amino acid–long protein with homology to T5bSS adhesins was demonstrated to be necessary for bacterial

adhesion to the surface of leaves, aggregation of bacteria, and incitation of host cell death (Rojas et al., 2002). On the basis of C-terminal extensions with homology to proteins toxic to bacteria, these putative T5SSs likely function to suppress competing bacteria rather than in virulence toward plants (Ruhe et al., 2013)

Type VI secretion system (T6SS) is the most recently described of the Gram-negative bacterial secretion systems and is widely distributed among diverse species. The T6SS has been shown to play an important role in pathogenicity towards eukaryotic host cells in a variety of important human pathogens, including *Pseudomonas aeruginosa, Burkholderia mallei, Vibrio cholerae* and *Aeromonas hydrophila* (English et al., 2014). It is now clear that the T6SS can be also used to target other bacteria and is thus likely to play an important role in polymicrobial infections. Various Type VI-secreted antibacterial toxins have recently been identified. The biggest group are peptidoglycan hydrolases including several families of peptidoglycan amidase and glycoside hydrolase enzymes which attack the cell wall of target bacteria (Russell et al., 2012; Whitney et al., 2013). Additionally, a superfamily of phospholipase effectors, attacking the target cell membrane, were described recently and a small number of other, unrelated, effector toxins have been experimentally identified such as antimicrobial toxins and phopsholipases, but they are yet to be fully characterized (Fritsh et al., 2013; Russell et al., 2013). In *P. atrosepticum*, the T6SS-encoding genes are induced in response to potato extracts (Mattinen et al., 2008). *P. wasabiae* SCC3193 has two putative T6SSs and a large inventory of putative tail and spike homologs (Nykyri et al., 2012). A mutant with deletions of unlinked genomic regions spanning 16 and 23 genes that included the two putative T6SS-encoding loci was modestly affected in its virulence on potato tuber slices (Nykyri et al., 2012). Pedron and colleagues (2014) revealed that *D. solani* encodes a distinctive arsenal of T5SS and T6SS related toxin-antitoxin systems.

Table 4. Secretion systems present in *Pectobacterium* and *Dickeya* genera. After Nykyri 2013

System	Function	Role in virulence	*Pectobacterium* spp.	*Dickeya* spp.
T1SS	Export of proteases, an adhesin and toxins.	Required for full virulence	*P. carotovorum* *P. c.* subsp. *brasiliense* *P. atrosepticum* (Marits et al., 1999; Pérez-Mendoza et al., 2011; Glasner et al., 2008)	*D. dadantii,* *D. solani* (Charkowski et al., 2012, Pedron 2014)

T2SS	Export of PCWDE, Svx protein and maybe other virulence determinants	Necessary for pathogenicity	All species (Toth et al., 2003; Glasner et al., 2008; Charkowski et al., 2012)	All species (Charkowski et al., 2012)
T3SS	Export of plant physiology modifying T3SS effectors.	Required for full virulence but T3SS is not present in all of the strains	*P. atrosepticum* *P.carotovorum* *P. carotovorum* subsp. *brasiliense* (Holeva et al., 2004; Pitman et al., 2009; Kim et al., 2009; Kim et al., 2011)	*D. dadantii* (Toth et al., 2005; Charkowski et al., 2012)
T4SS	Takes role in translocation of bacterial effectors directly to eukaryotic cytosol	Required for full virulence but T4SS is not widely present in the genus	*P. atrosepticum* *P. carotovorum* subsp. *brasiliense* (Bell et al., 2004; Glasner et al., 2008; Charkowski et al., 2012)	*D. solani* (Pedron 2014)
T5SS	Possibly secretion of large proteins such as serine protease, hemolysin and hemagglutinin. Bacterium-bacterium interaction (Cdi/Rhs)	Not investigated	*P. carotovorum* *P.carotovorum* subsp. *brasiliensis* *P. atrosepticum* *P. wasabiae* (Glasner et al., 2008; Poole et al., 2011)	All species (Rojas et al., 2002; Charkowski et al., 2012; Pedron et al., 2014)
T6SS	Export of unknown T6SS effectors	Required for full virulence and may be related to microbe-microbe interactions	*P. atrosepticum* (Liu et al., 2008; Mattinen et al., 2008)	*D. dadanti* (Koskiniemi et al., 2013) *D. solani* (Pedron et al., 2014)

3.5 Regulation

Virulence determinants in the SRE are controlled by complex regulatory networks, which act either positively or negatively on one (targeted regulation) or several (global regulation) determinants. They are stimulated by factors such as oxygen and nitrogen availability, temperature, pH, osmolarity, iron deprivation, growth phase, catabolite repression, plant degradation intermediates, plant extracts, DNA-damaging agents and very likely other factors yet to be identified. Newly identified regulatory proteins, thanks to a better understanding of the existing ones (Cui et al., 2001; Hyytiainen et al., 2001; Marits et al., 2002; Nachin and Barras, 2000; Nasser and Reverchon, 2002; Nguyen et al., 2002; Shih et al., 1999) continue to be added to the list of known regulators that have been reviewed extensively by Harris et al., 1998; Hugouvieux-Cotte-Pattat et al., 1996; Thomson et al., 1999. In Figure 5, there is presented the regulatory network scheme in *Dickeya* spp. For better understanding, the original explanation is presented under the figure (Charkowski et al 2012).

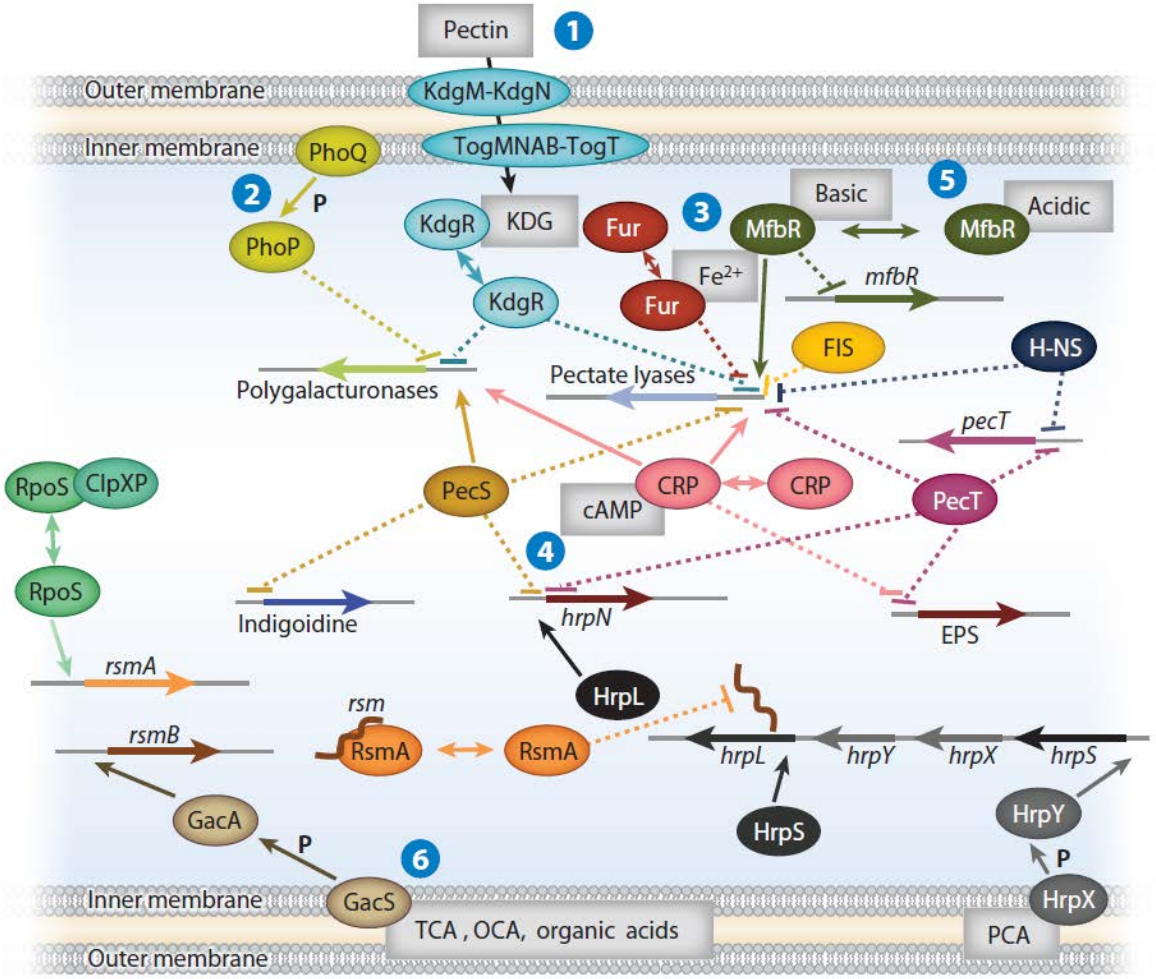

Figure 5. Model of pectinolysis/pathogenicity regulation in *Dickeya*.

Integration of small-molecule signals into regulation of exoprotein production. *Dickeya* exoprotein differs from *Pectobacterium* in that it does not rely upon AHL-mediated quorum sensing and that the horizontally acquired PecS, which is not present in *Pectobacterium*, is tightly integrated into virulence gene regulation. 1: Pectin is catabolized, fragments are imported through KdgMN and TogMNAG or TogT, and 2-keto-3-deoxygluconate (KDG) is produced: KdgR repression of pectate lyases is released, resulting in exoprotein expression. 2: Low magnesium levels are detected by the PhoPQ system and pectate lyase expression is repressed. 3: Iron levels are low in apoplastic fluids: The Fur-Fe^{2+} complex is dissociated and Fur repression of pectate lyases is released. 4: Glucose levels are low: The cAMP-CRP complex activates pectate lyase production. 5: Apoplast pH increases during infection: MfbR becomes active and activates pectate lyase production. 6: Organic acids are detected: GacAS represses *rsmA* and induces *rsmB*. The *rsmA* gene is also activated by the stationary phase sigma factor RpoS; RpoS is controlled in part by the protease ClpXP. The HrpXY two component system activates the HrpXY-HrpS-HrpL cascade and induces T3SS genes. Plant-derived organic acids that affect these regulatory systems include *o*-coumaric acid (OCA), p-coumaric acid (PCA), and *t*-cinnamic acid (TCA). Additional regulators directly control pectate lyase genes by interacting with their regulatory regions: The nucleoid-associated protein FIS represses plant cell wall–degrading enzyme (PCWDE) production during the early exponential phase. Disappearance of FIS in stationary phase results in induction of PCWDE genes. PecT and PecS are two pleiotropic repressors that control PCWDE and HrpN production as well as motility. In addition, PecS also controls indigoidine and other oxidative stress response genes, whereas PecT controls exopolysaccharide (EPS) production. PecT is itself under the tight control of the associated nucleoid protein H-NS. Abbreviation: P, phosphorylation. (Charkowski et al., 2012).

Three regulatory loci *kdgR, pecS,* and *pecT* have been identified that negatively control the expression of genes encoding pectinases in *Dickeya* spp. (Nasser et al. 1992; Praillet et al. 1996, 1997; Reverchon et al. 1991; Surgey et al. 1996). Among these regulators, the KdgR protein is the main effector and acts as a transcriptional repressor for all the genes coding for proteins involved in the pectin degradation pathway. In *D. dadantii* 3937, in the absence of pectic inducers, the KdgR protein binds to a specific 17-bp sequence (the KdgR box), which is conserved in the promoter region of the whole spectrum of genes encoding pectinases (Nasser et al. 1994). PecS negatively controls the production of pectinases and cellulases, as well as the production of the blue pigment indigoidine (antioxidant molecule), motility and the harpin HrpN (Reverchon et al., 1994; 2002; Rouanet et al., 2004; Nasser et al., 2005). PecT represses the production of pectinases, EPS, motility and the harpin HrpN (Surgey et al., 1996; Castillo and Reverchon, 1997; Condemine et al., 1999; Nasser et al., 2005). Pectinase production is also positively regulated. The cAMP receptor protein (CRP) activates the transcription of the pectinolysis genes (Nasser et al. 1997; Reverchon et al. 1997; Rouanet et al. 1999). H-NS protein (a protein that influences chromatin structure and gene expression in response to numerous growth parameters) plays an important role within the *pel* gene regulatory network (Nasser and Reverchon 2002). The studies have shown that H-NS is involved in the *D. dadantii* 3937 *pel* virulence pathway by exerting a negative effect on the expression of at least three regulatory loci: *expI*, *expR* and *pecT*, for example inactivation of H-NS results in an overproduction of PecT, which will in turn reduce the transcription of *pel* genes (Nasser and Reverchon 2002).

Work of our group (Potrykus et al., 2014a) revealed the role of several mentioned regulators in *D. solani* strains. Mutants of four *D. solani* strains were constructed by inactivating the genes coding either for one of the main negative regulators of *D. dadantii* 3937 virulence (*kdgR, pecS,* and *pecT*) or for the synthesis and perception of signaling molecules (*expI* and *expR*). An analysis of these mutants indicated that PecS, PecT, and KdgR play a similar role in both species, repressing, to different degrees, the synthesis of virulence factors. The thermoregulator PecT seems to be a major regulator of *D. solani* virulence (Potrykus et al. 2014a).

3.6 Identification and differentiation of SRE

The detection, identification and differentiation of SRE have been reviewed in the paper recently published by Czajkowski and colleagues (2015). Here, I present only a short description of methods used for those purposes.

3.6.1 Phenotypic methods

The most used worldwide medium for isolation of SRE is CVP medium (Crystal Violet Pectate) (Cuppels & Kelman, 1974; Hyman et al., 1998; Helias et al., 2012). Detection of SRE on CVP depends on the formation of characteristic deep cavities by the bacterial colonies. When the pathogen populations are low, they need to be enriched above detection level (Pérombelon, 2002). The tested material is incubated under anaerobic conditions in a liquid enrichment medium, PEB, containing sodium polypectate as the sole carbon source (Pérombelon & van der Wolf, 2002). The NGM medium was developed to differentiate *Dickeya* spp. from *Pectobacterium* spp. on the basis of the ability to produce the blue pigment – indigoidine (Lee et al., 2005).

In the past, biochemical tests were commonly used to differentiate strains of *Pectobacterium* spp. and *Dickeya* spp. from other bacteria. As the procedures are troublesome and time consuming, they tended to be replaced by more rapid serological and molecular methods (Czajkowski et al., 2015). Palacio-Bielsa and coleagues (2006) developed a modern version of biochemical test using a microtiter system. Sławiak and colleagues (2009) modified the method and checked features such as: growth at 39 °C, 41 °C and 25 °C on nutrient broth, anaerobic hydrolysis of arginine and polysaccharide inulin utilisation in phenol red peptone water (inulin extracts from chicory and dahlia were used at 0.3% final concentration); eight carbon sources were tested by acidification/alkalisation on liquid Ayers, Rupp and Johnson medium with bromothymol blue mixed with different 0.3% carbohydrates: (−)-D-arabinose, 5-keto-D-gluconate, mannitol, (+)-D-melibiose, (+)-D-raffinose and (−)-D-tartrate, β- gentiobiose and (+)-L-tartrate.

Other phenotypic methods used for distinguishing SRE are: fatty acid methyl ester analysis, volatiles profiling, serological methods, immunofluorescence staining and immunofluorescence colony staining (Czajkowski et al. 2015).

3.6.2 Genotypic methods

Molecular detection methods based on the analysis of bacterial genomic DNA have become most frequently used to detect and differentiate tuber soft rot and blackleg pathogens in environmental samples.

These are methods that can be applied to the mixture of bacterial strains and allow detection and identification of pathogens present in symptomatic or symptomless plant samples. Several single PCR and multiplex PCR assays with species specific primers have been developed for the detection of soft rot and blackleg pathogens; multiplex PCR assays can simultaneously detect a few kinds of pathogens in the tested sample. In our laboratory the multiplex PCR assay was developed which detects and distinguishes SRE from *P. c. subsp. carotovorum* / *P. wasabiae*, *P. atrosepticum* and *Dickeya* spp. (Potrykus et al., 2014b). Real-time PCR procedures have also been developed for detection of SRE (Brierley et al., 2008; Laurila et al., 2010; Kim et al., 2011, Pritchard et al., 2012; Van der Wolf et al., 2014a). Pritchard and colleagues (2012) presented a bioinformatic tool allowing an easy prediction of primer sets for specific detection of species and subspecies of bacteria based of the raw genome sequence information.

On the other hand, there are more sophisticated methods for differentiantion of closely related strains but they require isolation of pure bacterial culture. Restriction fragment length polymorphism of amplified PCR (PCR-RFLP) product (usually one of the housekeeping genes) may be applied specifically for the identification and differentiation of closely related isolates within species and subspecies. To differentiate between *Pectobacterium* and *Dickeya* species, two groups of genes were mainly used; those coding for virulence factors (for example *pel* genes by Darrasse et al., 1994) and the housekeeping genes (e.g. 16S rDNA, 23S rDNA, *recA, gyrA, gyrB, rpoS* and *dnaX*). Waleron and colleagues (2002ab) developed a PCR-RFLP assay to differentiate pectinolytic *Pectobacterium* and *Dickeya* species on the basis of the housekeeping genes *recA* and *rpoS*.

Multilocus sequence analysis (MLSA) using concatenated sequences of the intergenic spacer (IGS), as well as *dnaX, recA, dnaN, fusA, gapA, purA, rplB, rpoS* and *gyrA* have been used in the study of *Dickeya* genus regarding the establishement of *Dickeya solani* species (Van der Wolf et al., 2014b). This study revealed that the group of *D. solani* is homogenous and distinct from other *Dickeya* species (Figure 6).

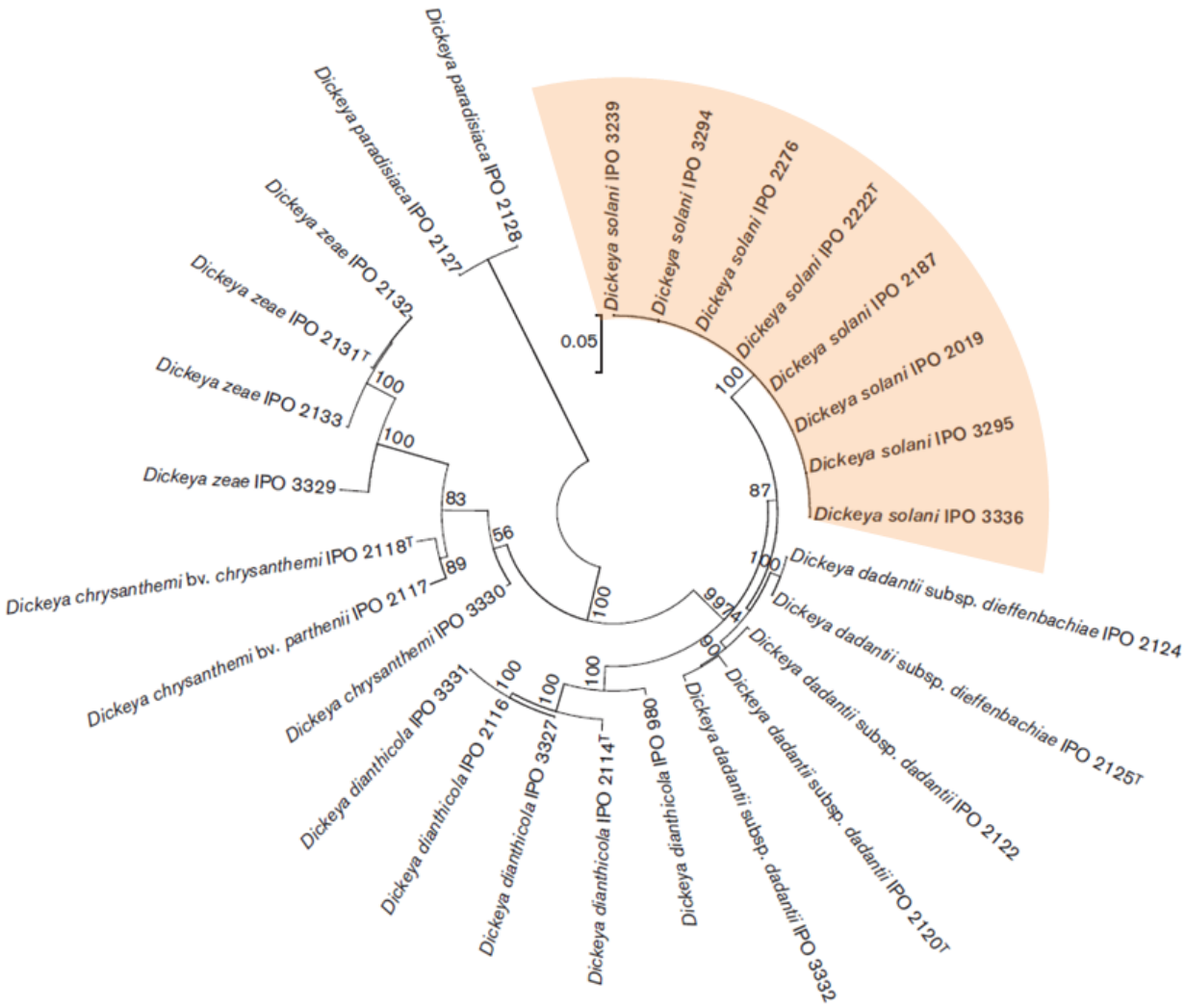

Figure 6. Bootstrap consensus tree of concatenated sequences of *dnaX, dnaN, fusA, gapA, gyrA, purA, rplB, rpoS, recA* and IGS of 27 members of the genus *Dickeya* belonging to different species (Van der Wolf et al., 2014).

The methods mentioned above have been developed on the basis of single genes, but there are also methods applying a whole-genome approach such as rep-PCR or PFGE and, of course, genomes sequencing which are described below.

Repetitive sequence-based PCR (rep-PCR) has been developed to target the repetitive sequences present in bacterial genomes, namely Repetitive Extragenic Palindromic elements (REP), Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR) and BOX elements (BOX-PCR) (Versalovic et al., 1991 and 1994). The relative resolution of the generated

patterns is high and allows phylogenetic classification of SRE from the genus down to strain level. The REP-PCR analyses were used for example in studies on the classification of a new clade of *Dickeya* spp. biovar 3, *D. solani* (Slawiak 2009b, Degefu et al., 2013) and to characterise different *Pectobacterium* and *Dickeya* species in potato in South Africa and Zimbabwe (Ngadze et al., 2012). The technique is easy to perform and its resolution is high.

Macrorestriction analysis by pulsed-field gel electrophoresis (PFGE) has been used successfully for strain differentiation of plant pathogenic bacteria (Egel et al., 1991; Grothues & Rudolph, 1991; Zhang & Geider, 1997). PFGE protocol (Ribot et al., 2001) with modifications was successfully used for the analysis of PFGE profiles of *E. chrysanthemi*, demonstrating that PFGE could easily differentiate white-flowered calla lily isolates of *E. chrysanthemi* from those from other plants and could be used for epidemiological studies (Lee et al., 2006). PFGE was also used to discriminate *Pectobacterium* spp. clades in diseased stems and tubers of potato plants originating from different fields in Korea (Kim et al., 2009). PFGE was also successfully applied to confirm the identity and homogeneity of *D. solani* strains isolated in Finland and Israel (Degefu et al., 2013; Tsror et al., 2013).

The development of next generation sequencing (NGS) techniques let the scientists delve into more detailed search for differences among the strains of the same or different species (Toth et al., 2003; Pritchard et al., 2012). Complete or draft genome sequences are now available for numerous SRE phytopathogens, with many more in draft formats. In the National Center for Biotechnology Information (NCBI) genomes database already 53 genomes assemblies of *Pectobacterium* spp. and 41 genome assemblies of *Dickeya* spp. are available (http://www.ncbi. nlm.nih.gov/). A typical member of this group has a single circular chromosome of circa 5 Mb and no large plasmids are present. Comparative genomics can reveal physiological and functional variation among bacteria that provides insight into their ability to exploit distinct ecological niches (Toth et al., 2006). Genome sequences provide a valuable resource for discovery of molecular markers that can be used for reliable classification of prokaryotic taxa and for understanding evolutionary relationships among them (Lerat et al., 2005; Dutilh et al., 2008; Gupta, 2010; Bhandari et al., 2012; Gao & Gupta, 2012). The complete genome sequence of *D. dadantii* 3937 has been published (Glasner et al. 2011) as well as genomes of 7 *D. solani* strains at different stages of assembly (Garlant et al., 2013, Pritchard et al., 2013a, Khayi et al., 2014; Golanowska et al., 2015).

The term ‘pangenome’ denotes the set of all genes present in the genomes of all members of a group of organisms, usually a species (Tettelin et al., 2005). The pangenome includes genes present in all genomes of the group (known as the core genome), genes in the genomes of a few members of the group (so-called accessory genome) and genes that are present in only one organism of the group (known as unique genome). Tettelin and colleagues (2005) used the genomes of eight strains of *Streptococcus agalactiae* to characterize the pangenome and concluded that it is ‘open’ (meaning that the pangenome has an infinite size) and that each new added genome would provide, on average, 33 new genes. These authors first established orthologous groups of genes, which allowed the designation of a core genome (the genes present in all strains) of 1 806 genes (~80 % of each individual genome) and showed that 95 % of these core genes share greater than 90 % identity.

3.7 Importance of the research on *Dickeya spp.*

Potato (*Solanum tuberosum* L.) is among the top ten commodities produced in the world. In 2013 it was on the sixth position in the top 10 commodities (FAOSTAT, http://faostat3.fao.org/home/E; Figure 7). The first reported disease caused by *E. chrysanthemi* (most probably belonging to *D. dianthicola*) on potato in Europe occured over 40 years ago (Maas Geesteranus, 1972). In most European countries, losses attributable to SRE have remained generally low, but in the recent years, potato losses caused by *Dickeya* spp. have increased significantly in a number of European countries and in Israel (a major importer of European potato seed tubers) (Figure 8, Toth et al., 2011). Up to 25% of the potato blackleg incidences in the Netherlands, Belgium and France have been attributed to infections by *Dickeya* spp. (Toth et al. 2011). This may be associated with the emergence of a new *Dickeya* species – *D. solani* - that is most likely spread by trade in seed tubers and, potentially in future years, could have a larger impact as a consequence of climate change (Toth et al., 2011).

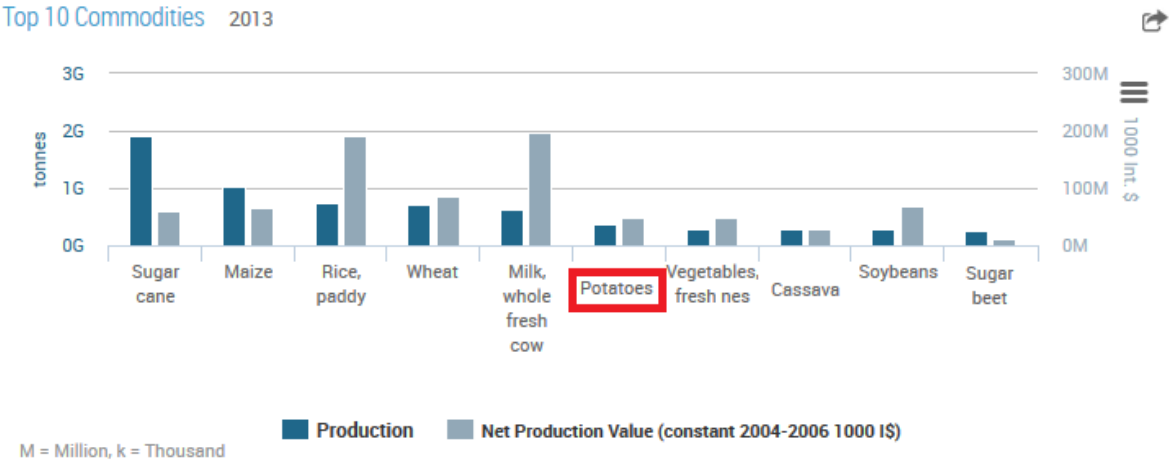

Figure 7. Ten top commodities produced in the World. FAOSTAT 2013.

Until 2000, *P. atrosepticum* and *D. dianthicola* were considered to be responsible for the majority of potato blackleg infections in Europe (Perombelon and Kelman, 1980; Perombelon, 2002). In general, *Dickeya* spp., are high-temperature pathogens, and were considered to play a major role in blackleg epidemiology in tropical and subtropical regions. Over the past 10 years in Europe, the incidence of infections of seed potato with bacteria from the genus *Dickeya*. increased relatively in comparison to those with *Pectobacterium* spp. (Laurila et al., 2008; Lojkowska et al., 2010; Toth et al., 2011).

Dickeya solani strains are considered to be more aggressive than other blackleg causing bacteria. Preliminary analysis suggested that they need lower optimal temperatures for disease development as well as lower inoculum levels for infection spreading. They seem to have a greater ability to colonize potato plants roots and to spread through the plants' vascular system (Toth *et al.* 2011, Czajkowski *et al.* 2012). In 3 years of field studies in the Netherlands with *D. dianthicola* and *D. solani*, disease detection varied annually and between strains. In summary, Czajkowski and colleagues (2012) concluded that *D. solani* possesses features which allow more efficient plant colonization than *D. dianthicola*.

The results of biochemical assays, rep-PCR and 16S rDNA and *dnaX* sequence analyses indicated that all strains of *D. solani* characterized so far show 100% of homology, which could suggest a common origin, possibly a single introduction in the potato ecosystem (Sławiak et al., 2009b).

There is little information about *D. solani* existence outside European countries and Israel. There is no evidence of *D. solani* presence in Americas or Australia. But there is a notification of *D. solani* presence in China on hiacynth bulbs (Chen et al., 2015) and in

Malaysian waterfalls (GenBank accession number JSXD00000000.1, Win-Si Tan, University of Malaysia). *D. solani*, however, was not only isolated from symptomatic potato plants. It was isolated from potato rhizosphere as a potential biocontrol strain against *Rhizoctonia solani*, *Verticillium dahliae* and *Phytophthora infestans* from rhizosphere in the field study of genetically modified potatoes in Germany (Weinert et al., 2010). This strains possessess identical rep-PCR and RFLP-PFGE profile as the Type Strain of *D. solani* IPO 2222 and strains isolated in Poland in 2005 and 2009 (IFB 0099 and IFB 0158). However, they indicated a much lower ability to macerate potato tissue and a lower ability to produce PCWDE (Potrykus et al 2014a).

Apart from availability of 7 *D. solani* genomes, the genetics of *D. solani* is poorly understood in comparison to what is available and known about *D. dadantii* 3937. The genomic analyses on *D. solani* strains performed by Garlant and colleagues (2013) and Pedron and colleages (2014) have revealed open reading frames (ORFs) possibly involved in virulence and the production of toxic compounds. They were annotated as polyketide synthases (PKS), non-ribosomal peptide synthetases (NRPS) and amino acid adenylation domain proteins, suggesting that they encode proteins that maybe involved in the production of such metabolites as antibiotics, toxins. Pedron and colleagues (2014) pointed out a higher viariability in proteins transported *via* T5SS/T6SS in *D. solani* 3337 than in *D. dadantii* 3937.

The knowledge about regulation of the genes expression encoding virulence factors in *D. solani* has been obtained in our laboratory (Potrykus et al., 2014a). The main information about virulence, regulation is acquired from the knowledge about closely related and mostly studied species – *D. dadantii* 3937. This strain's metabolism and regulation of pectinolysis have been studied for about 40 years (Kotoujanski et al., 1982). A lot of tools have been developed for *D. dadantii* 3937, so the extention of knowledge about the features and pathogenicity strategy of SRE can be better understood. Nowadays, by means of comparative and functional genomics, researchers can improve the understanding of adaptation modes to different ecological niches and the genes that determine SRE pathogenicity (Toth et al., 2006).

There is a need for intense research on the molecular regulation of the pathogenicity of *D. solani*, not only because of its fast spreading across Europe or better accommodation to various climatic conditions (not only tempeate climates such as in Poland or cold in Finland, but also hot climates such as in Israel), but also because of the lack of efficient means to fight the soft rot and blackleg diseases during the vegetation and storage.

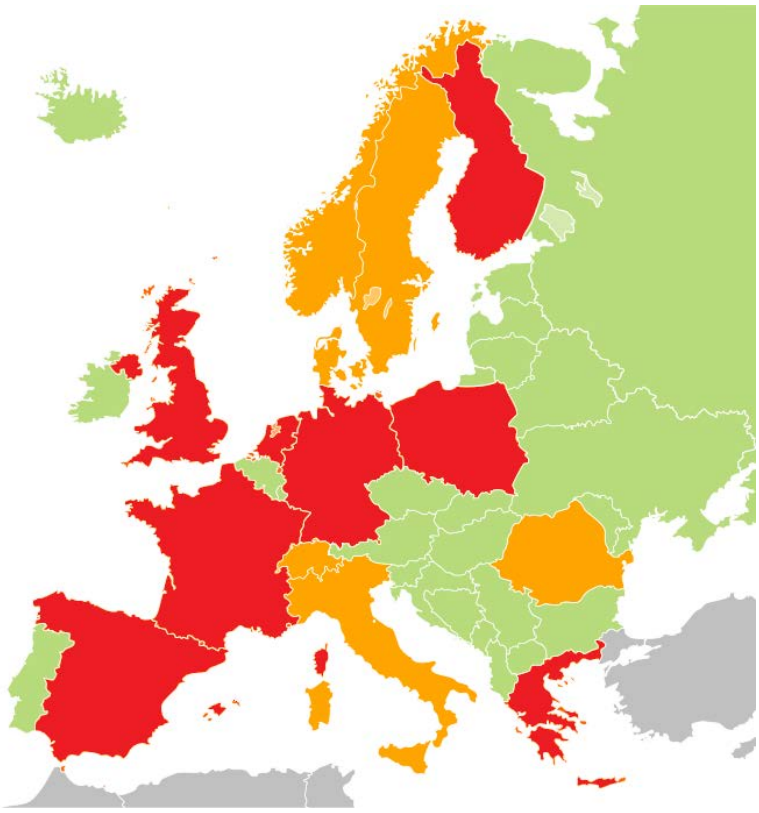

Figure 8. Presence of *D. solani* and *D. dianthicola* species in Europe in 2011. After Toth et al. 2011. Red colour indicates presence of *D. solani*, orange *D. dianthicola*.

4 Aims of the study

1. Phenotypic and genotypic characterization of the *D. solani* strains isolated in countries with different climatic conditions: Poland, Finland and Israel,

2. Study of the potato tuber extract influence on the expression of a few selected *D. solani* genes: *pelD, pelL, tssK, lfaA,*

3. Comparative genomics of ten *D. solani* strains, performed on 4 genomes sequenced for this study (two highly virulent and two low virulent strains) and 6 genome sequences available in the GenBank databases.

5 Materials

5.1 Bacterial strains

Table 5. *Dickeya* sp. Wild Type, Type Strains

Name	IFB Collection number (other collections)	Country and year of isolation	Source
Dickeya dadantii	IFB 0010 [a,b] (NCPPB 898T, IPO2120)	Comoros 1960	*Pelargonium capitatum*
Dickeya dadantii	IFB 0016 [c] (3937, A4922)	France	*Sepolia ionantha*
Dickeya dianthicola	IFB 0103[a] (NCPPB 453T, IPO2114)	UK 1956	*Dianthus caryophyllus*
Dickeya dianthicola	IFB 0157[d] (LMG28825)	Poland, 2009	*Solanum tuberosum*
Dickeya dianthicola	IFB 0188[a] (IPO1741)	Netherlands, 1992	*Solanum tuberosum*
Dickeya solani	IFB 0123[a] (IPO2222)	Netherlands, 2007	*Solanumtuberosum*
Dickeya solani	IFB 0099[d] (IPO2276, LMG28824)	Poland 2005	*Solanumtuberosum*
Dickeya solani	IFB 0100[d] (IPO2277)	Poland 2005	*Solanum tuberosum*
Dickeya solani	IFB 0158[d] (LMG 28826)	Poland, North 2009	*Solanum tuberosum*
Dickeya solani	IFB 0167[d]	Poland, South-west 2009	*Solanum tuberosum*
Dickeya solani	IFB 0212[d]	Poland, Central 2010	*Solanum tuberosum*
Dickeya solani	IFB0221[e]	Germany	Potato rhizosphere
Dickeya solani	IFB0223[e]	Germany	Potato rhizosphere
Dickeya solani	IFB 0231[f] (VIC-BL 25)	Finland	*Solanum tuberosum*
Dickeya solani	IFB 0236 [f] (VIC-SRI 5)	Finland	*Solanum tuberosum*
Dickeya solani	IFB 0254[f] (SRG 5-4)	Finland	*Solanum tuberosum*
Dickeya solani	IFB 0261[f] (BLG 4-5)	Finland	*Solanum tuberosum*
Dickeya solani	IFB 0265[f] (SRG 54-4)	Finland	*Solanum tuberosum*
Dickeya solani	IFB 0124[a] (IPO3228)	Israel, 2008	*Solanum tuberosum*
Dickeya solani	IFB 0125[a] (IPO3296)	Israel, 2008	*Solanum tuberosum*
Dickeya solani	IFB 0455[g] (IPO3204)	Israel, 2007	*Solanum tuberosum*
Dickeya solani	IFB 0456[g] (IPO3213)	Israel, 2008	*Solanum tuberosum*
Dickeya solani	IFB 0457[g] (IPO3236)	Israel, 2009	*Solanum tuberosum*

[a]-J.Wolf, PRI, Wageningen, Holland, [b]- Toth, SCRI, Dundee, Scotland, [c]- N. Hugouvieux, MAP, INSA, Lyon, Francja, [d]- LPPB, Intercollegiate Faculty of biotechnology UG and MUG, Gdansk Poland, [e] - K. Smalla, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany, [f]- Yeshetila Degefu, Finland, [g] – Leah Tsror, Israel.

Table 6. *Dickeya* spp. mutants

	Collection Number IFB/MAP	**Species**	**Genotype**	**Source**
Mutants in genes encoding pectate lyases	IFB 0460 A 4415	*D. dadantii*	*pelD*::*uidA* kan^R	MAP, INSA, Lyon
	IFB 0462 A 5595	*D. solani*	IFB0099 *pelD*::*uidA* cm^R	transduction, this work
	IFB 0463 A 5596	*D. solani*	IFB0123 *pelD*::*uidA* cm^R	transduction, this work
	A1945	*D. dadantii*	A350 *pelZ*::*uidA* kan^R	Jafra et al., 1999
	A5670	*D. dadantii*	A4922 *pelZ*::*uidA* kan^R	transduction, this work
	A3003	*D. dadantii*	*pelI*::*uidA* kan^R	Jafra et al., 1999
	A4417	*D. dadantii*	*pelN*::*uidA* kan^R	Hassan et al.,2013
	IFB 0465 A5298	*D. dadantii*	*pelL*::*uidA* kan^R	Jafra et al., 1999
	IFB 0466 A 5687	*D. solani*	IFB0099 *pelL*::*uidA* kan^R	transduction, this work
	IFB 0467 A 5688	*D. solani*	IFB0123 *pelL*:: *uidA* kan^R	transduction, this work
Mutants in genes encoding structures of Type VI Type Secretion System	IFB 0468 A 5594	*D. dadantii*	A4922 *tssK*::*uidA* cm^R	recombination, this work
	IFB 0469 A 5587	*D. dadantii*	A4922 *tssK*::*uidA* kan^R	recombination, this work
	IFB 0470 A 5603	*D. solani*	IFB0099 *tssK*::*uidA* cm^R	transduction, this work
	IFB 0471 A 5604	*D. solani*	IFB0123 *tssK*::*uidA* cm^R	transduction, this work
Mutants in LacI regulators family	IFB 0472 A 4391	*D. dadantii*	*lfaA*::*uidA* kan^R	Van Gijsegem et al., 2008
	IFB 0473 A 5631	*D. dadantii*	A4922 *lfaA*::*uidA* cm^R	recombination, this work
	IFB 0474 A 5637	*D. solani*	IFB0099 *lfaA*::*uidA* cm^R	transduction, this work

	IFB 0475 A 5638	*D. solani*	IFB0123 *lfaA*::*uidA* cm^R	transduction, this work
	IFB 0480 A 4959	*D. dadantii*	*lfaT*::*uidA* kan^R	Van Gijsegem et al., 2008
	IFB 0481 A 5704	*D. solani*	IFB0099 *lfaT*::*uidA* kan^R	transduction, this work
	IFB 0482 A 5705	*D. solani*	IFB0123*lfaT*::*uidA* kan^R	transduction, this work

Other bacterial strains:

Salmonella enterica serotype Braenderup (strain H9812, ATCC) – standard in PFGE.

Escherichia coli NM522 – used in transformation.

5.2 Plasmids

Table 7. Plasmids used in the study.

Name	Genotype	Source
pGEM		Promega Corporation, USA
I 1224	pN715 (*pelD*) amp^R	MAP, INSA, Lyon
I 3031	pGEM *lfaA::*amp^R	Van Gijsegem et al., 2008
I 3100	CKC17 cm^R	MAP, INSA, Lyon
I 3580	pGEM *tssK::* amp^R	this work
I 3581	pGEM *tssK*::*uidA* kanR amp^R	this work
I 3634	pI3634 *pelD*::*uidA* cmR amp^R	this work
I 3635	pI3635 *tssK*::*uidA* cmR amp^R	this work
I 3636	pI3636 *lfaA*::*uidA* cmR amp^R	this work

5.3 Phages

Table 8. Phages used in the study

Name	Genotype	Source
ØEC2	Wild Type	Resibois et al., 1984
ØEC2/ A5586	*pelD*::*uidA* cm^R	this work
ØEC2/ A5594	*tssK*::*uidA* cm^R	this work
ØEC2/ A5631	*lfaA*::*uidA* cm^R	this work
ØEC2/ A5298	*pelL*::*uidA* kan^R	this work

5.4 Genomic sequences

Genomic sequences of the 10 *D. solani* strains were analyzed. Four sequences from strains IFB 0099, IFB 0158, IFB 0221, IFB 0223 were obtained at LPPB and six collected from GenBank (NCBI) source. All sequences were analyzed with *D. dadantii* 3937 reference genome (GenBank accession no. CP002038, Glasner et al. 2011).

Table 9. Genomic sequences used in the study

Abbreviation used in the figures	*D. solani* strains	Country of isolation	Source	GenBank accession number
IFB0099	IFB 0099 (IPO 2276)	Poland	potato	JXRS00000000 Golanowska et al. 2015
IFB 0158	IFB 0158	Poland	potato	unpublished
IFB 0221	IFB 0221	Germany	potato rhizosphere	unpublished
IFB0223	IFB0223	Germany	potato rhizosphere	unpublished
IFB 0123	IFB 0123 (IPO2222)	Netherlands	potato	AONU00000000 Pritchard et al. 2013
GBBC2040	GBBC2040 (LMG25865)	Belgium	potato	AONX00000000 Pritchard et al. 2013
MK10	MK10	Israel	potato	AOOP00000000 Pritchard et al. 2013
MK16	MK16 (IPO 3494)	Scotland	river water	AOOQ00000000 Pritchard et al. 2013
Ds0432	D s0432-1 (IFB 0135)	Finland	potato stem	AMWE00000000 Garlant et al. 2013
RNS or IPO 3337	RNS 08.23.3.1A (IPO3337)	France	potato	AMYI00000000 Khayi et al. 2014

5.5 Media

5.5.1 Luria Broth (LB)/ Luria Agar (LA)

Composition (1000 ml):

Bactotryptone	10 g
Yeast extract	5 g
NaCl	10 g
NaOH	to adjust pH
Agar	12 g

All compounds were dissolved in 1000 ml of distilled water and pH was adjusted to 7. The medium was autoclaved at 120 °C for 20 min.

5.5.2 Tryptone Soya Broth / Agar (TSB / TSA)

Composition (1000 ml):

Tryptone	15 g
Soytone	5 g
NaCl	5 g
Agar	15 g

All compounds were dissolved in1000 ml of distilled water and pH was adjusted to 7-7.2. The medium was autoclaved at 120 °C for 20 min.

5.5.3 M63

Composition (1000 ml):

KH_2PO_4	13.6 g (0.1 M)
$(NH_4)_2SO_4$	2 g (15 mM)
$FeSO_4$	0.5 mg (9 µM)
KOH 6M	to adjust pH
$MgSO_4$	0.2 g (1 mM)
Vitamin B1	1 mg
Agar	15 g

KH_2PO_4, $(NH_4)_2SO_4$ and $FeSO_4$were added to 800 ml of distilled water, pH was adjusted with KOH to 7.0. $MgSO_4$solution and vitamin B1, were added to the final volume of 1000 ml. The medium was autoclaved at 120 °C for 20 min (optional 110°C, 20 min).

5.5.4 M63 Y/ M63 Y agar

To prepare M63 Y 3g of glycerol per 1000 ml was added to M63 medium. For solid medium 15 g of agar was added per 1000 ml.

5.5.5 M63 Y PGA /M63 Y PGA agar

Medium M63 Y was supplemented with poligalacturonic acid (PGA) 4 g per 1000 ml. For solid medium 15 g of agar was added per 1000 ml.

5.5.6 M63 Y CMC / M63 Y CMC agar

Medium M63 Y was supplemented with carboxymethylcellulose (CMC) 10 g per 1000 ml. For solid medium 15 g of agar was added per 1000 ml.

5.5.7 M63 Y PE

Medium M63 Y was supplemented with plant extract to the final concentration of 1 % v/v.

5.5.8 Milk agar

Composition (1000 ml):

Nutrient broth	30 g
Agar	15 g
Milk 0% fat	100 ml

Nutrient broth and agar were added to 900 ml of distilled water and autoclaved at 120 °C for 20 min. Milk was boiled and added to the solution after autoclaving, stirred and poured on Petri dishes.

5.5.9 Soft top agar

Composition (1000 ml):

Bactotryptone	10 g
Yeast Extract	5 g
NaCl	10 g
NaOH	to adjust pH
Agar	4 g

Powders were dissolved in distilled water, pH was adjusted to 7-7.2 with NaOH and autoclaved at 120 °C for 20 min.

5.5.10 LGC agar (Kretshmer et al. 1975)

LA was supplemented with glucose 2g per 1000 ml and $CaCl_2$ to the final concentration of 2 mM.

5.5.11 CVP (Helias et al. 2012)

Composition (1000 ml):

- Crystal violet solution (500 ml of hot distilled water):

$CaCl_2$ x $2H_20$	1.02 g
tryptone	1 g

$Na_3C_6H_5O_7$	5 g
$NaNO_3$	2 g
Agar	6 g
Crystal violet 0.1 % aqueous solution	1.5 ml

- Pectin solution (500 ml of hot distilled water):

NaOH 5M	2 ml
Pectin (Dipecta, Agdia-Biofords, France)	18 g

The crystal violet solution and the pectin solution were prepared and autoclaved separately before being mixed together. Ingredients of both solutions were introduced sequentially in the same order as the component lists of the recipe. Each ingredient of the crystal violet solution, except the agar, was dissolved by stirring the medium before adding the following one. The water for pectin solution was heated to 80–100 °C before NaOH and pectin were added, to allow the pectin to dissolve under stirring. After autoclaving (120 °C for 15 min, both solutions) and while still hot, the crystal violet solution was slowly poured into the pectin solution, while gently stirring the medium using a magnetic bar to avoid bubble formation. The final pH was verified to be 7.

5.5.12 Chrome – Azurol - S agar (CAS agar, Schwyn and Neilands, 1987)

Composition (1000 ml):

- Dye solution:

CAS	60.5 mg in 50 ml of water
$FeCl_3$ x $6H_2O$	10 ml of a 1 mM solution in 10 mM HCl
CTAB	72.9 mg in 40 ml of water

- Agar solution:

KH_3PO_4	3 g
NaCl	0.5 g
NH_4Cl	1 g
$MgSO_4$ x $7H_2O$	0.2 g
Glucose	4 g
Agar	15 g

- Casamino acids — 30 ml of 10% stock solution
- $CaCl_2$ — 10 ml of stock solution 0.01M

To prepare 1000 ml of the medium, 60.5 mg CAS was dissolved in 50 ml water and mixed with 10 ml iron(III) solution (1 mM $FeCl_3$, x $6H_20$, 10 mMHCl). This solution was slowly added to 72.9 mg CTAB dissolved in 40 ml water (under stirring). The resultant dark blue liquid was autoclaved (120 °C, 15 min). Ingredients of agar solutions were solubilized in 850 ml of Tris-HCl

buffer pH 6.8. Agar solution was adjusted to pH 6.8 and also autoclaved. After cooling to 50 °C 30 ml of sterile casamino acids and 10 ml of sterile $CaCl_2$ were added. The dye solution was finally added along the glass wall, with slow agitation to achieve mixing without generation of foam.

5.6 Antibiotics

5.6.1 Ampicillin stock solution 10 mg/ml (Fluka)

Stock solution was diluted to 100 µg/ml final solution.

5.6.2 Kanamycin stock solution 5 mg/ml (Fluka)

Stock solution was diluted to 50 µg/ml final solution.

5.6.3 Chloramphenicol stock solution 2 mg/ml (Fluka)

Stock solution was diluted to 20 µg/ml final solution.

5.7 Buffers/Solutions

5.7.1 Calcium chloride solution ($CaCl_2$) I

0.2 M solution was autoclaved at 115 °C for 20 min.

5.7.2 Calcium chloride solution ($CaCl_2$) II

0.5 M solution was autoclaved at 115 °C for 20 min.

5.7.3 PGA solution

2.5% of polygalacturonic acid (Sigma) solution in water. Autoclaved at 120 °C for 15 min.

5.7.4 Thiourea solution

1 M solution.

5.7.5 Tris-HCl 1M pH 8.0

121.1 g of Tris was dissolved in 700 ml of distilled water and 60 ml of HCl (36 %), pH was adjusted to 8.0. Volume was adjusted to 1000 ml. Solution was autoclaved at 120 °C for 20 min and later stored at room temperature.

5.7.6 EDTA 0.5 M pH 8.0

186.1 g of EDTA was dissolved in 800 ml of distilled water, pH was adjusted with NaOH to 8.0 and the volume was adjusted to 1000 ml. The solution was autoclavedat 120 °C for 20 min and later stored at room temperature.

5.7.7 TE

Solution containing 10 mM Tris and 1 mM EDTA, pH adjusted to 8.0.

5.7.8 Cell suspension buffer

Solution containing 100 mM Tris pH 8.0 and 100 mM EDTA, pH adjusted to 8.0, sterilized by filtration through 0.2 µm filter.

5.7.9 Cell lysis buffer

Solution containing 50 mM Tris pH 8.0, 50 mM EDTA pH 8.0, 1% of N-lauroylsarcosine and proteinase K (0.1 mg/ml final concentration). Sterilized by filtration through 0.2 µm filter.

5.7.10 TAE x 50

Composition (1000 ml):

Tris base	242 g
Glacial acetic acid	57.1 ml
0.5 M EDTA pH 8.0	100 ml

Tris was dissolved in 700 ml of water, and EDTA and acetic acid were added, pH was adjusted to 8.0 (with HCl), and volume was adjusted to 1000 ml with distilled water. Solution was stored at room temperature. Dilution 1 : 50 was used as final solution.

5.7.11 TBE x 5

Composition (1000 ml):

Tris base	54 g
Boric acid	27.5 g
0.5 M EDTA pH 8.0	20 ml

Tris was dissolved in 700 ml of water, and EDTA and boric acid were added, pH was adjusted to 8.0 (with HCl), and volume was adjusted to 1000 ml with water. The solution was stored at room temperature. Dilution 1: 10 was used as final solution.

5.7.12 PL Buffer – for evaluation of pectate lyases specific activity

Composition (100 ml):

1M Tris, pH 8.5	10 ml
Sterile distilled water	88 ml
0.2 M $CaCl_2$	50 µl
2.5% PGA	2 ml

10 ml of Tris was added to 88 ml of sterile distilled water. $CaCl_2$ and PGA were added in given order as sterile solutions (autoclaved at 120 °C, 15 min). The solution was stored at 4 °C. Before use the buffer was warmed up to 37 °C.

5.7.13 P-nitrophenyl B-D-glucuronide (PNPU) 10mM – substrate for evaluation of GUS specific activity

31 mg of PNPU was dissolved in 10 ml of M63.

5.7.14 Low-phosphate buffer

Composition (100 ml):

$Na_3C_6H_5O_7$	0.5 g
$MgSO_4$ x 7 H_2O	0.1 g
$(NH_4)_2SO_4$	1 g
KCl	0.15 g
KH_2PO_4	0.068 g
Tris	12 g

Compounds were dissolved in distilled water, pH was adjusted to 7.5. The solution was autoclaved at 115 °C for 20 min.

5.7.15 Sodium Dodecyl Sulfate 10%

100 g of sodium dodecyl sulfate (SDS) was added to water and mixed, volume was adjusted to 1000 ml. The solution did not need to be autoclaved. The solution was stored at room temperature.

5.7.16 Sol A

Composition (100 ml):

0.5 M glucose	10 ml
0.5 M EDTA pH 8.0	2 ml
1M Tris pH 8.0	2.5 ml
H_2O	85.5 ml

The solution was autoclaved for 15 min at 120 °C and later stored at 4 °C.

5.7.17 Sol B

Composition (10 ml):

10N NaOH	0.2 ml
10% SDS	1 ml
H_2O	8.8 ml

The solution was stored for a week at room temperature.

5.7.18 Sol C

Composition (100 ml):

5 M potassium acetate	60.0 ml
glacial acetic acid	11.5 ml
H_2O	28.5 ml

The solution was stored at 4°C.

5.7.19 Restriction enzymes

Restriction enzymes used in this work:

- AfeI, BamHI, EcoRI, EcoRV, HindIII, PstI (New England Biolabs)
- Sma, XbaI (Fermentas)

5.7.20 PCR primers

Table 10. PCR primers used in the study

Name of the product/gene	Sequence
REP1 R1 (Versalovic et al. 1991)	CGGICTACIGCIGCI I I I
REP 2-1 (Versalovic et al. 1991)	ICGICTTATCIGGCCTAC
ERIC-1R (Versalovic et al. 1991)	ATG TAA GCT CCT GGG GAT TCA
ERIC-2 (Versalovic et al. 1991)	AAG TAA GTG ACT GGG CTG AGC
BOXX (Versalovic et al. 1994)	CTA CGG CAA GGC GAC GCT GAC

tssK forward (this work)	GCTCTAGATCACCGATGTGGCGCTGG
tssK reverse (this work)	CACGGGATCCTCCTGTTAACTAC
lfaA forward (Van Gijsegem et al. 2008)	GCGGATCCATTGAAACATTGGGTATTGC
lfaA reverse (Van Gijsegem et al. 2008)	GCTCTAGATCCTGTAACAGGCAGG

5.7.21 PCR reagents

REP - PCR reactions were performed with the use of Fermentas reagents (Stock solutions: PolimeraseTaq 5U/ul, buffer for polimerase 10x concentrated, $MgCl_2$ solution 25 mM, dNTP's solution 10 mM).

Illustra™ PuReTaq Ready To Go PCR Beads (GE Healhcare) were used according to producers protocol for tssK and lfaA PCR products amplification.

5.7.22 Agaroses

- Basica LE GQT, Prona Agarose
- SeaKem Gold Agarose, Lonza Inc.

5.8 Commercial kits

- A&A Genomic Mini Genomic DNA isolation kit.
- Macherey Nagel NucleoSpin Tissue Genomic DNA isolation kit.

5.9 Laboratory equipment

5.9.1 Electrophoresis equipment

- Pulse Field Gel Electrophoresis – CHEF-DR III Variable Angle System, BioRad Laboratories, Inc. USA
- Mupid-One Advance Co. Ltd.

5.9.2 Thermocyklers

- C1000 Touch Thermal Cycler, BioRad Laboratories Inc.
- PCR-EXPRESS-Thermal-CyclerHYBAID

5.9.3 Gel visualization equipment

- Gel Doc, BioRad Laboratories, Inc. USA

- Transvisualisator, UVP Inc.

5.9.4 Spectrophotometers

- DU-640 Beckman Coulter, USA
- Specord 205, Analytic Jena, Germany
- EnVision, Multilabel Plate Reader, Perkin Elmer, USA

5.10 Computer software

- Bio Edit sequence alignment editor, version 7.1.3.0 (Hall, T.A. 1999)
- Biophyton
- Celera assembler (version 8.0)
- ContiGUATOR (Galardini et al. 2011)
- DuctApe (Galardini et al. 2014)
- FastQC(version 0.10.1).
- LS-BSR (Sahl et al. 2014)
- Statistica 12, StatSoft INC. USA
- Quantity One, Bio Rad Laboratories, Inc. USA
- seq_crumbs package (version 0.1.8)
- StreamingTrim (version 1.0).
- pacBioToCA
- prokka (version 1.7.2)
- runCA program

5.11 Databases

- GenBank, National Center for Biotechnology Information (NCBI), USA
- ASAP, University of Wisconsin, USA

6 Methods

6.1 Strain isolation and cultivation

Strains of different *Dickeya* species other than *D. solani* were obtained from several international Culture Collections (Table 5). Polish *D. solani* isolates were obtained in 2005, 2009 and 2010 in the routine procedure of isolating pectinolytic bacteria from infected potato tissue (both plant shoots and tubers) according to the procedure established in LPPB IFB UG-MUG laboratory (Potrykus et al. 2014b).The infected potato tissue was homogenized with a hand held homogenizer (Bioreba, Switzerland) in sterile Bioreba filtered bags with phosphate buffer and then spread on CVP (Materials 5.5.11). Pectinolytic bacteria were later analyzed with a PCR multiplex reaction designed for distinguishing three different groups of pectinolytic bacteria (*P. c.* subsp. *carotovorum* / *P. wasabiae, P. atrosepticum* and *Dickeya* spp.).

Five *D. solani* strains from Finland were obtained by the courtesy of Dr. Yeshitila Degefu, MTT Agrifood Research Finland, Biotechnology and Food Research, Agro Biotechnology University of Oulu, FI, Finland. Two Israeli strains were obtained by the courtesy of Dr. Jan van der Wolf, Plant Research International, Wageningen, the Netherlands. Three Israeli strains were obtained by the courtesy of Dr. Leah Tsror, Gilat Research Center, Israel.

The bacteria were maintained in LB, LA or CVP media (Materials 5.5.1 and 5.5.11) at 28 °C if not otherwise stated. All tested bacterial strains were stored in 40 % glycerol at -80 ºC.

6.2 *D. solani* strains growth rate determination

Determination of growth rate was established in 48-well plates with the use of EnVision spectrophotometer plate reader (Perkin Elmer, USA) with orbital shaking at 28 and 37 °C. The night culture of a strain was refreshed 1 : 50 in fresh LB medium, the final volume of the culture was 500 µl. The measurement was performed every hour for 20 h.

6.3 Phenotypic characterization of *D. solani* strains from different climate conditions

6.3.1 Pectinases activity assay (Reverchon et al. 1986)

The test was performed on M63Y PGA plates (Materials 5.5.5). 2 µl of bacterial suspension containing 10^8 CFU/ml was spotted on a plate and incubated for 48h. Later on, the plates were covered with 10 % copper acetate. Copper acetate reacts with PGA, giving the blue color of medium. If bacteria degrade PGA there is a whitish halo around bacterial colonies. The halo diameter is in proportion to the activity of produced pectinases. The experiment was performed at three different temperatures: 18, 28 and 37 °C, and repeated three times.

6.3.2 Cellulases activity assay (Andro et al. 1984)

The test was performed on plates with M63Y CMC (Materials 5.5.6). 2 µl of bacterial suspension containing 10^8 CFU/ml was spotted on a plate and incubated for 48 h. Later on the plates were covered with 2 % Kongo Red and after a few minutes solution of 5 M sodium chloride was added. If bacteria are able to degrade CMC, an orange halo is observed around bacterial colonies. The halo diameter is in proportion to the activity of the produced cellulases. The experiment was performed at three different temperatures: 18, 28 and 37 °C, and repeated three times.

6.3.3 Proteinases production

The test was carried out on milk agar plates (Materials 5.5.8). 2 µl of bacterial suspension containing 10^8 CFU/ml was spotted on a plate. The halo diameter was measured after 48 h of incubation. The experiment was performed at three different temperatures: 18, 28 and 37 °C, and repeated three times.

6.3.4 Ability to chelate iron (Schwyn and Neilands 1987)

The test was carried out on CAS plates (Materials 5.5.12). 2 µl of bacterial suspension containing 10^8 CFU/ml was spotted on a plate. The halo diameter was measured after 72 h of incubation. The experiment was performed at three different temperatures: 18, 28 and 37 °C, and repeated three times.

6.3.5 Motility assays (Harshey et al. 2003)

The assays were performed on Luria Agar (Materials 5.5.1) media with different agar concentrations, 0.3% (3g/L) for swimming motility assay and 0.8% (8g/L) for swarming motility. 2 µl of bacterial suspension containing 10^8 CFU/ml was spotted on a plate, then incubated for 48 h. Bacterial growth was observed after 24 and 48 h. The experiment was performed at three different temperatures: 18, 28 and 37 °C, and repeated three times.

6.3.6 Maceration ability test

Potatoes of Lord variety were bought at the local supermarket. Tubers were washed carefully under running water and sterilized with 10% of commercially available bleach (i.e. ACE or Clorox) for 20 minutes. Later on, the tubers were thoroughly washed and left in water for another 10 minutes for rinsing out the bleach. Potatoes were dried under the laminar flow until the skin was dry. Dried tubers were cut crosswise into 10 mm thick slices. Slices were placed in glass Petri dishes containing a layer of Whatman filter paper. Depending on the slice size 2 or 3 holes 5 x 5 mm were cut out. Into each hole 50 µl of bacterial suspension was added. Paper was hydrated with 5 ml of sterile water. Potato slices were inoculated with three different inolulum levels (10^5, 10^6 and 10^7 CFU/ml of the suspension). The inoculated slices were incubated at 28 °C for 48 h. The diameter of rotten tissue was measured. The experiment was repeated twice.

6.4 Genetic characterization of *D. solani* strains from different climate conditions

6.4.1 REP profiling (Versalovicet al., 1991 and 1994)

Polish, Finish and Israeli strains of *D. solani* and the Type Strains of different species of *Dickeya* (Table 5. *Dickeya* sp. Wild Type, Type Strains) were analysed using the repetitive sequence – based PCR with two pairs of primers: ERIC 1R and ERIC 2 (Enterobacterial Repetitive Intergenic Consensus), REP 1 R-I and REP 2-I (Repetitive Extragenic Palindromic) and also BOX1AIR as described by Versalovic et al. in 1991 and 1994 respectively (Materials 5.7.20). Genomic DNA was purified using Genomic Mini AX Bacteria Kit (Materials 5.8).

PCR amplifications were performed in an automated thermal cycler (BioRad Laboratories Inc. USA) with an initial denaturation (95 °C, 7 min) followed by 30 cycles of denaturation

(90 °C, 30 sec), annealing (REP, 40 °C, 1 min; ERIC, 52 °C, 1 min, BOX, 53 °C, 1 min), and extension (65°C, 8 min) with a single final extension (65 °C, 16 min).

The resulting amplification products were separated by electrophoresis in 0.8 % agarose (Basica, Prona) gel in a 0.5 x TBE buffer at 50 Volts for 2.5 h and visualized and documented using the Gel Doc imaging system (Bio Rad Laboratories Inc.) supported by Quantity One Software (Bio Rad Laboratories Inc. USA).

6.4.2 Pulse Field Gel Electrophoresis(Lee et al. 2006)

All strains of *D. solani* and Type strains of *D. dadantii* and *D. dianthicola* were analyzed by PFGE method. *Dickeya* sp. cells were grown for 24 h on TSA medium (Materials 5.5.2). Genomic DNA for PFGE was prepared in agarose plugs using the method described by Ribot and colleagues (2001) with modifications. The cells were suspended in cell-suspension buffer (Materials 5.7.8) to an optical density OD_{600} of 2.0 measured with a DU 640 Spectrophotometer (Beckman). Bacterial suspensions were mixed with 20 µl of proteinase K (20 mg ml^{-1}; A&A Biotechnology, Poland) and an equal amount of 1 % SeaKem® Gold agarose (Lonza Inc.) in TE buffer (10 mm Tris, 1 mm EDTA, pH 8.0) and pipetted into a plastic mould. The hardened agarose plugs were immersed in cell lysis buffer (Materials 5.7.9) for 2 h at 56 °C in shaking water bath. After lysis, the plugs were washed (10 min per wash) at 56 °C three times with sterile double-distilled water and three times with TE buffer (Materials 5.7.7) in a shaking water bath. Chromosomal DNA plugs were incubated with the restriction enzyme Xba1 (Fermentas) at 37 °C for 2 h. Restriction fragments were separated by PFGE through 1% SeaKem® Gold agarose in 0.5 x TBE buffer (Materials 5.7.11) containing 100 mM thiourea (Materials 5.7.4) with a CHEF-DR III chiller system (BioRad, Laboratories Inc., USA) using 0.5 x TBE buffer as a running buffer. PFGE was performed at field strength of 200 V for 22 h at 14°C, with the pulse time linearly increased from 2 to 54 s and a fixed reorientation angle of 120°C. After the electrophoresis run was completed, the gels were stained with ethidium bromide solution (0.5 µg ml^{-1}), distained in water, and the band pattern was observed under UV illumination (GelDoc BioRad Laboratories Inc., USA). As a standard *Salmonella enterica* serotype Braenderup (strain H9812, ATCC) (Materials 5.1) prepared according to the protocol was used.

6.5 Genomes analysis

6.5.1 Sequencing

Four strains from our Laboratory collection were chosen for whole genome sequencing, namely strains IFB 0099, IFB 0158, IFB 0221 and IFB 0223. Two strains were chosen because they indicated high pathogenicity (IFB 0099, IFB 0158) and two others (IFB 0221, IFB 223) because they indicated a lower pathogenicity level than the type strain of *D. solani* (IPO 2222). The goal was to analyse the 4 mentioned genomes and other 6 *D. solani* genomes available at the time of analysis and the genome of the reference strain *D. dadantii* 3937. Genomes of trains IFB 0099 and IFB 0223 were sequenced by 454 Roche technology and re-sequenced by PacBio technology. Genomes of the IFB 0158 and IFB 0221 were sequenced by Illumina Paired-End DNA Sequencing.

6.5.2 Assembly and annotation

Strains IFB0099 and IFB0223 have been assembled using a hybrid assembly procedure, combining 454 and PacBio reads using the Celera assembler (version 8.0). 454 reads have been converted to the FastQ format using the seq_crumbs package (version 0.1.8) and trimmed using StreamingTrim (version 1.0). The qualityof both 454 and PacBio reads has been checked using FastQC (version 0.10.1). The PacBio reads coverage was found to be high enough to perform a hybrid assembly (IFB0099: 80X, IFB0223: 72X), using the pacBioToCA and the runCA program. The best assembly parameters have been checked using the Quast web server.

Strains IFB0158 and IFB0221 have been assembled from Illumina Paired-Ends reads using Abyss (version 1.3.6). The quality of the reads has been checked using FastQC (version 0.10.1) and the first 9 bases of each reads, which showed poor quality and high k-mer repetitions, have been trimmed using the seq_crumbs package (version 0.1.8). The Abyss assembly has been carried out using a value of k equal to 64, 32 and 16, choosing the assembly with k=64, which produced the highest number of contigs and the highest N50. All four strains have been annotated using prokka (version 1.7.2), as well as strains IPO 2222, MK10, MK16 and RNS, whose annotation was not yet available in GenBank at the time of analysis.

6.5.3 Orthology assesment

The orthology relationships inside the *D. solani* pangenome have been computed using the Blast-BBH algorithm implemented in the DuctApe suite (version 0.16.2).

6.5.4 Phylogeny

The six strains (IFB0099, IFB0223, IFB0158, IFB0221, RNS and D s0432-1) for which no scaffold information was available have been aligned to the *D. dadantii* 3937 strain using the CONTIGuator web server (version 2.7.3; e-value 1e-5, hit length threshold 250, contig coverage threshold 5%). A pairwise genomic alignment of 10 scaffolds (IFB0099, IFB0223, IFB0158, IFB0221, RNS and D s0432-1, MK 10, MK16, GBBC2040 and IPO2222) has been computed through megablast (version2.2.28+), keeping hits over 5000 bp; the alignment has been visualized using the genome diagram package inside BioPython (version 1.62b).

6.5.5 LS-BSR (Sahl et al. 2014)

LS-BSR is a method to compare all coding regions in a large set of genomes. Each peptide is compared against its nucleotide sequence in order to obtain the maximum BLAST bit score. Each peptide is then aligned against each genome in order to find the query BLAST bit score. The query divided by the reference provides one with the BSR, which can range from 0 to 1; scores slightly higher than 1.0 can be observed due to variable bit scores obtained by BLAST.

6.6 Construction of the *D. solani* mutants

6.6.1 Preparation of construct for *E. coli* transformation

PCR reaction was performed with the use of designed primers (Table 7). Illustra™ PuReTaq Ready To Go PCR Beads (GE Healhcare, Materials 5.7.21) were used according to producers protocol for tssK and lfaA PCR products amplification.

PCR amplifications were performed in an automated thermal cycler (Hybaid PCR Express) with an initial denaturation (95 °C, 5 min) followed by 35 cycles of denaturation (95 °C, 1 min), annealing (tssk 50 °C, 2 min; lfaA 62 °C,2 min), and extension (72 °C, min) with a single final extension (72 °C, 10 min).

6.6.2 Ligation to pGEM-T vector

PCR products (of the genes chosen for modification) were ligated to pGEM-T plasmid vector (materials 5.2).

Standard procedure for ligation to pGEM-T plasmid: 5 µl of PCR product was added to 3.5 µl of ligase buffer. Next 0.5 µl of pGEM-T vector was added and 1 µl of ligase. Ligation mix was incubated at 15°C per night. 5 µl was used for transformation of *E. coli* NM522.

6.6.3 Preparation of *E. coli* NM522 competent cells

0.5 ml of a *E. coli* NM522 night culture of was put in fresh 100 ml of LB (Materials 5.5.1) and incubated with agitation at 37 °C until OD_{600} reached 0.5. Then the suspension was divided in portions of 40 ml and put into centrifuge tubes and incubatedfor 10 minutes on ice. The cultures were centrifuged at 4 °C for 10 minutes, 7 000 rpm and the supernatant was removed. The pellet was suspended in ½ volume of cold 100 µM $CaCl_2$ (20 ml) and incubatedfor 20 min on ice. Again, samples were centrifuged at 4 °C for 10 minutes, 7000 rpm. Then again, the supernatant was removed and the pellet was suspended in 2 ml of cold 100 µM $CaCl_2$ and 1.2 ml 40% glycerol. The suspensions were mixed by pipetting and 200 µl portions were put into prepared cold Eppendorf tubes. The cells were stored at -80 °C.

6.6.4 Transformation of *E. coli* NM522

5 µl of ligation mixture was added to 200 µl of competent cells and kept on ice for 20 min. Then, the sample was incubated for 2 min at 42 °C (heat shock) and 5 min on ice again. 800 µl of LB was added and the cells were incubated at 30 °C from 2 up to 4 h with mixing by inversion from time to time. After incubation 100 µl of suspension was spread on LA plate (Materials 5.5.1) containing the required antibiotic and the rest was centrifuged for 5 min at 4000 rpm and pellet was suspended in 200 µl of LB and also spread on another plate with the required antibiotic (Materials 5.6).

6.6.5 Isolation of plasmid DNA from *E. coli* NM522

1 ml of the tested strain night culture was centrifuged for 2 min, 12000 rpm. The supernatant was removed, the pellet suspended in 100 µl of cold Sol A (Materials 5.7.16). 200 µl of Sol B (room temperature) (Materials 5.7.17) was mixed by inversion. 150 µl of cold Sol C (Materials 5.7.18) was added, mixed by inversion of the tube then centrifuged for 5 min at

12000 rpm. The supernatant was collected to the already prepared tubes containing 1 ml of 100% ethanol, mixed and centrifuged for 10 min/12000 rpm, then, the supernatant was removed, 1 ml of 70% ethanol was added. Then again the sample was centrifuged for 2 min/12000 rpm and afterwards the supernatant was removed. The sample was dried and then resuspended in 50 µl of sterile water.

6.6.6 Digestion of the plasmid

According to the producer's protocol of the restriction enzyme (Materials 5.7.19)

6.6.7 Inactivation of enzymes and precipitation of DNA

The sample after digestion was inactivated by the following procedure: 70 µl of water was added to the sample and incubated for 10 min at 68 °C. 10 µl Sol C (Materials 5.7.18) and 250 µl of EtOH 100% was added to the sample and centrifuged for 5 min 12 000 rpm, the supernatant was removed and 500 µl of 70 % EtOH. The sample was centrifuged 5 min / 12 000 rpm and dried.

6.6.8 Preparation of *D. dadantii* electrocompetent cells

Five ml of night preculture was put into 100 ml of LB liquid medium (Materials 5.5.1) and incubated at 40 °C for 5.5 h with shaking. Then, the suspension was divided into two 50 ml cornings and centrifuged 6500 rpm/ 6 min/ 20 °C. The supernatant was removed and the pellet resuspended in 50 ml of sterile water in room temperature (together in one corning). The sample was centrifuged for 7000 rpm/ 8min/20 °C, the supernatant was removed. The pellet was resuspended in 50 ml sterile water (room temperature) again centrifuged 7000 rpm/8mins/20 °C andthe supernatant removed. The pellet was resuspended in 2 ml of 10 % glycerol and put 50 µl per Eppendorf tube and stored at -80 °C.

6.6.9 Electroporation of *D. dadantii*

1 µl of plasmid was added to the previously prepared electrocompetent cells and transferred to electropotator cuvettes with 2 mm diameter. After electroporation, 1 ml of LB (Materials 5.5.1) was added, transferred to Eppendorf tube and incubated at 30 °C for 2 h. After incubation 200 µl of bacterial suspension was spread on LA plates (Materials 5.5.1) containing the required antibiotic (Materials 5.6). The rest of bacterial suspension was centrifuged for 5 min, 4000 rpm, the supernatant was removed, and the pellet was resuspended in 100 µl of LB and spread on the same medium.

6.6.10 Preparation of phage stock (Resibois et al. 1984)

200 µl of a donor strain night culture (*D. dadantii* with the desired mutation) was added to cold 200 µl of preexisting EC2 phage suspension in a glass tube (Materials 5.3). The mixture was incubated at room temperature without agitation. After incubation 5 ml of liquid soft top agar (Materials 5.5.9) was added, vortexed and immediately poured on LGC plate (Materials 5.5.10) and left to condense. The plate was incubated for 16 h at 30 °C. After incubation, agar overlay was resumed by using 5 ml of LB (Materials 5.5.1) and an inoculation spreader. The liquid was recovered to a centrifuge tube, a few drops of chloroform were added and the suspension was vortexed until it reached homogeneity. Later the tube was centrifuged for 15 min, 6000 rpm and the supernatant was transferred to a glass tube with a few drops of chloroform. The tube was vortexed again and left for at least 15 min at 4 °C before use. Phage stock was stored at 4 °C.

6.6.11 Transduction to *D. solani*

200 µl of *D. solani* night culture was added to 200 µl phage stock suspension in a glass tube. The mixture was incubated for 20 min at room temperature without agitation. 0.8 ml of LB (Materials 5.5.1) was added and incubated between 4 to 6 h at 30 °C with agitation. The suspension was then transfered into sterile Eppendorf tube and centrifuged for 2 min, 8000 rpm. The supernatant was removed and the pellet was resuspended in 100 µl of LB and spread on LA plate (Materials 5.5.1) containing the required antibiotic (Materials 5.6). The plates were incubatedfor 48 h at 30 °C.

6.6.12 Confirmation of the correctness of the obtained mutants

The obtained clones were verified for the presence of mutation by PCR reaction with the use of PCR beads according to the producer protocol (primers Materials 5.7.20, beads Materials 5.7.21). The result was observed after agarose gel electrophoresis in 1 % of agarose gel (Basica, Prona) in 1 x TAE as running buffer (Materials 5.7.10). The bands were visualized after ethidium bromide staining for 15 min under UV illumination (GelDoc BioRad Laboratories Inc, USA).

6.6.13 Bacterial cell extract preparation for PL and GUS activities determination

A preculture of a desired strain or mutant in M63Y (Materials 5.5.4) was incubated for 24 h at 28 °C. A second preculture in M63Y was made (100 µl from previous preculture was added to the fresh medium) and again incubated 24 h at 28 °C. For enzymatic activity determination,

the third culture was made in non-induced and induced conditions for each strain/mutant (M63 Y – non-induced conditions Materials 5.5.4, M63 Y supplemented with 0.2 % PGA Materials 5.5.5; or 1 % plant extract Materials 5.5.7) and incubated for 24 h at 28 °C. Then the OD_{600} was measured. To 1 ml of each culture a few drops of toluene were added and vortexedfor 30 s and incubatedat 4 °C for at least 3 h. Before further use the toluene was evaporated under the fume hood. Prior to the pectate lyases enzymatic assay, the samples were centrifuged for 2 min, 12 000 rpm. Prior to GUS activity assay, the samples were not centrifuged.

6.6.14 Potato tubers extracts preparation

The crude extract of potato tubers (PE) was obtained by crushing and extraction with a juicer. The extracts were then centrifuged twice for 5 min at 12000 rpm to eliminate the largest cellular debris. They were then filtered through tissue-paper filters and then through Millipore filters RC 0.45 and 0.2 µm to remove the remaining microparticles and bacteria. The extracts were stored at -20 °C. The crude extracts were added to the culture medium with a dilution of 100 x which corresponds to a final concentration of 1% in M63 Y (vol / vol).

6.6.15 Pectate lyase activity test (PL) (Tardy et al. 1997)

One unit of pectate lyases activity was defined as the amount of the enzyme required to produce 1 µmol of unsaturated product per minute. The assays were performed on extracts of bacterial cultures (Methods 6.6.13). Total pectate lyases activity was expressed as µmoles of unsaturated products (UP) liberated per 1 min per 1 mg of bacterial dry weight.The measurement was performed using spectrophotometer (Specord 205, Analytic Jena, Germany) at 235 nm, at 37 °C, during 5 min. The molar extinction coefficient of UP is Ɛ = 5200. Given that 0.28 mg dry weight of bacteria (PSB) corresponds to an OD 600 0.6, the total pectatelyasesactivityis calculated by the formula (in µmol of unsaturated oligomers formed per min per mg PSB):

$$AS\ PL = C_{UP} \times m_{PSB}^{-1} = 0.412 \times \Delta OD_{235} \times \text{dilution of the sample} \times OD_{600}^{-1}$$

6.6.16 GUS activity test (Bardonnet and Blanco, 1992)

The activity assay β-glucuronidase (GUS) is based on the cleavage of *p*-nitrophenyl- β -D-glucuronide (PNPU) to nitrophenol (PNP) measured at 405 nm. The assays were performed on extracts of bacterial cultures (Methods 6.6.13). The molar extinction coefficient of PNP is Ɛ = 4500. The activity of β -glucuronidase is expressed in nmol PNP liberated per 1 min per 1

mg of bacterial dry weight). The measurement was performed using spectrophotometer (Specord 205, Analytic Jena, Germany) at 405 nm, at 37 °C, during 5 min. The activity of glucuronidase is calculated by the formula (in nmol PNP formed per min and per mg PSB):

$$AS\ GUS = C_{PNP} \times m_{PBS}^{-1} = 476.2 \times \Delta OD_{405} \times \text{dilution of the sample} \times OD_{600}^{-1}$$

7 Results

Bacteria from the genus *Dickeya* cause disease symptoms especially in tropical and subtropical climates and host plants including potato, maize and banana among many other crops. However, in recent years potato losses caused by *Dickeya* spp. have increased significantly in a number of European countries and in Israel (the latter a major importer of European potato seed tubers). The emergence and spread of *D. solani*, together with the effects of climate change, cause more losses on potato platations in temperate climate. *D. solani* strains were shown to cause more severe losses than *D. dianthicola*, *P. atrosepticum* and *P. carotovorum* subsp. *carotovorum* (Toth et al., 2011). Soft rot erwinias tend to out-compete other bacteria in tuber rots because of their ability to produce larger quantities of a wider range of cell wall-degrading enzymes. For the disease development very important are factors such as faster growth, PCWDE production (pectinases, cellulases, and proteases), motility, that facilitate ability to macerate potato tissue.

The question: why *D. solani* is more aggressive that the other SRE species has been raised by the researchers across Europe. Studies show that they are able to induce diseases at lower inoculum levels, they produce more PCWDE and they possess an arsenal of T5SS/T6SS secreted proteins of many more functions than destroying the plant cell-wall. An interesting issue is that *D. solani* is able to cause severe disease symptoms in a wide range of climates (for example in northern Europe countries like Finland through the Netherlands, Poland, France, Switzerland to Asian country such as Israel). There is little comparative study on *D. solani* strains originating from different climate conditions, but it is known, that they cause more and more severe losses on potato plantations.

Here, I ask a question if and how temperature influences the fitness of the strains originating from different climatic zones, because there is evidence that strains originating from different climatic zones differ in their ability to cause disease symptoms. I was trying to answer this question by studying several phenotypic and genotypic traits of strains originating from Poland, Finland and Israel, countries with different climatic conditions.

A part of my study was performed in the Laboratory of Microbiology, Adaptation and Pathogenesis at INSA, Lyon, under the supervision of my co-promoter Dr. Nicole Hugouvieux-Cotte-Pattat. I constructed mutants in *D. solani* strains in several genes whose

expression could be influenced by plant derivatives, in order to find plant signal(s) responsible for the induction of the expression of genes encoding virulence factors.

Another part of the study, performed in collaboration with Prof. Marco Bazzicalupo's group from the University of Florence, Florence, Italy, was dedicated to comparative genomics of 10 available *D. solani* genome sequences, including strains of high and low virulence, to elucidate the genomic factors that could be responsible for differences in their virulence.

7.1 Characteristics of *D. solani* strains from different climate zones

The purpose of this part of study was to compare strains originating from different climatic conditions and elucidate if the origin of the strains has an influence on their virulence. The strains chosen for the study were isolated in countries with different climate conditions – Poland, Finland and Israel. According to Koeppen classification (http://www.fao.org/nr/climpag/globgrids/kc_classification_en.asp, Pirdwiny 2006): Poland and Finland have cold moderate climate (though they differ in the matter of summer, in Poland the summer is moderate and in Finland it is short and cold) while Israel has hot, desert climate. Several phenotypic and genotypic tests were applied to elucidate the importance of different factors responsible for the aggressiveness of the strains. The growth of the tested strains was studied in 2 temperatures (28 and 37 ºC). Their ability to produce PCWDE as well as their ability to cause potato maceration, iron accommodation and motility were tested at three different temperatures (18, 28 and 37 °C). After the analysis of the results from plate-based tests, 28 ºC was chosen as the optimal temperature for total pectinolytic activity and potato maceration evaluation. The total pectinolytic activity was measured in non-inducing and inducing conditions of the pectate lyases activity. The potato maceration test was performed using three levels of inoculums (10^5, 10^6 and 10^7 CFU/ml) in order to allow observation of the differences between strains' ability to macerate potato tuber tissue.

The strains for this part of the analysis were chosen on the basis of their origin and strain availability in IFB Laboratory Collection. Five strains from each climatic group were characterized and compared. Strains of 3 *D. solani* groups were compared with *D. solani* Type Strain (IFB 0123, IPO 2222), *D. dadantii* (Type Strain - IFB 0010 and IFB 0016 known

as 3937) and *D. dianthicola* (Type Strain - IFB 0103; IFB 0157 and IFB 0188) reference strains; all strains are listed in Materials, Table 5.

To analyze the influence of temperature and species on the phenotypic traits of *Dickeya* spp. strains, the two-way ANOVA followed by post-hoc Tukey test were applied. Later, theinfluence of temperature was analyzed for strains of the same origin and differences between them were described. A statistical analysis (two-way ANOVA followed by Tukey post-hoc test) of the influence of temperature and the origin of *D. solani* strains originating from different climatic conditions was performed.

7.1.1 Analyses of phenotypic features of *Dickeya* strains using qualitative tests

For all tested features presented below (besides the analysis of growth), the following procedure has been applied.

1) Analysis of *D. dadantii* (2 strains: IFB 0010 –*D. dadantii* TS and IFB 0016– the 3937 strain which is the most studied strain of the related species), *D. dianthicola* (3 strains: IFB 0103 – *D. dianthicola* TS; IFB 0157 – originating from Poland and IFB 0188 – originating from the Netherlands) and *D. solani* TS (IFB 0123). The chosen strains represent the *Dickeya* species known as a threat to potato plantations. *D. dadantii* is a known potato pathogen in hot climates, *D. dianthicola* is is a pathogen causing soft rot in temperate climates and *D. solani* is a newly emerged pathogen spreading across Europe.
2) Strains of *D. solani* originating fom different climatic zones have been compared in terms of the potential influence of their origin on the expressed traits.
3) Traits of *D. solani* groups of the strains coming from Poland, Finland and Israel (5 strains in each group) were analyzed statistically to evaluate the influence of temperature of incubation and the origin of the strains on the tested features. Traits such as an ability to produce pectinolytic, cellulolytic, proteolytic enzymes as well as motility and iron ions chelation ability were studied in three different temperatures (18, 28 and 37 °C). Statistical analysis compromised two-way ANOVA test of variance followed by post-hoc Tukey test.

7.1.1.1 Influence of temperature on Dickeya spp. growth

The growth curves for all strains from different climates as well as reference strains were determined with the use of EnVision spectrophotometer during 20 h. Due to the lack of possibility for performing the measurement at 18 °C (equipment requirements) the experiment was conducted only at 28 and 37 °C. The mean value of two experimental replicates was analyzed and shown in Figures 9 and 10 (for the graphs clarity in this case the error bars werenot included). The groups of 5 *D. solani* strains originating from different climatic conditions were compared to *D. solani* ^{TS}, *D. dadantii* and *D. dianthicola* strains. Later, the strains were compared within each group with each other and Type Strain. The results were gathered in the summarizing Table 11.

All of the tested strains grow at 28 and 37 °C and the growth curves are presented in Figures 9 and 10. The value for each strain of the time at which it reaches the stationary phase and the corresponding optical density (OD_{600}) is presented in Table 11.

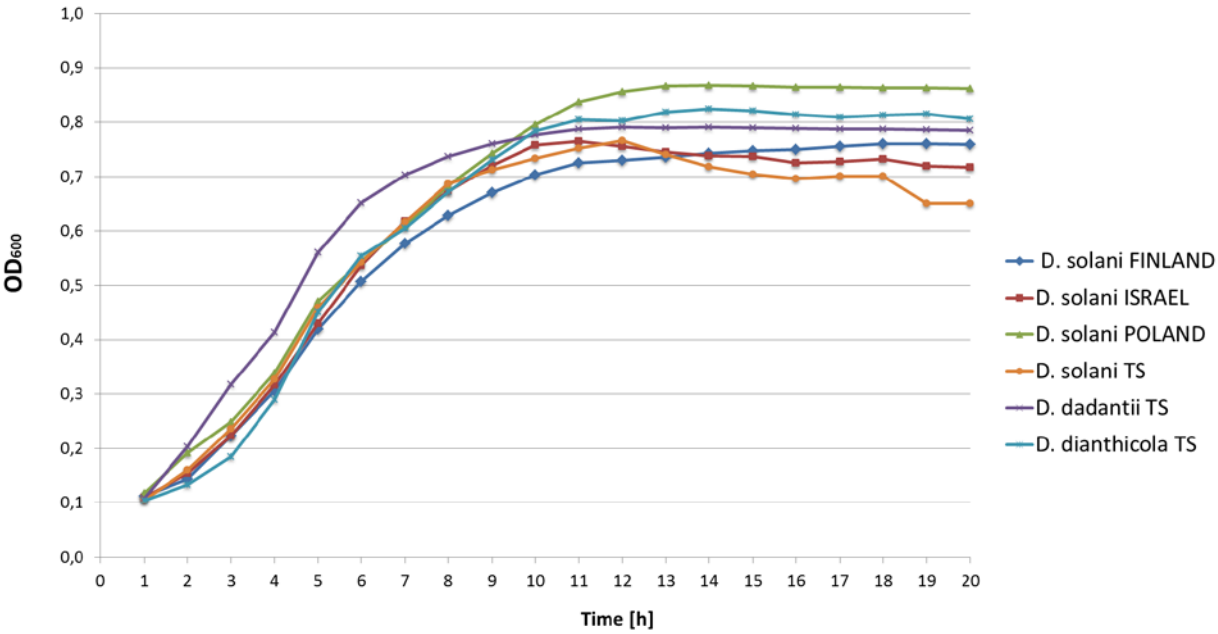

Figure 9. Comparison of growth curves of *D. solani, D. dadantii* and *D. dianthicola* strains at 28 °C.

At 28 °C, all the tested *D. solani* strains have a similar growth dynamic. Polish *D. solani* strains reach the highest density, but the difference is not statistically significant (one-way ANOVA, $p>0.05$). The *D. solani*^{TS} strain reaches the stationary phase after the same time as *D. dadantii*^{TS} (after 12h), earlier than Polish, Finnish, Israeli and *D. dianthicola* ^{TS} strains

(13.8 h, 15.6 h, 12.8 h and 14 h, respectively). In general, all of the strains grow similarly. The statistical analysis did not show significant differences between the tested strains.

At 37 °C, the growth of *D. solani* strains is more diverse than at 28 °C. At this temperature *D. dianthicola*^{TS} reaches the stationary phase the fastest (after 12 h), earlier than *D. dadantii* (13 h) and *D. solani* (TS – 16 h, Polish – 14.8 h, Finnish – 15.4 h and Israeli strains – 16 h).

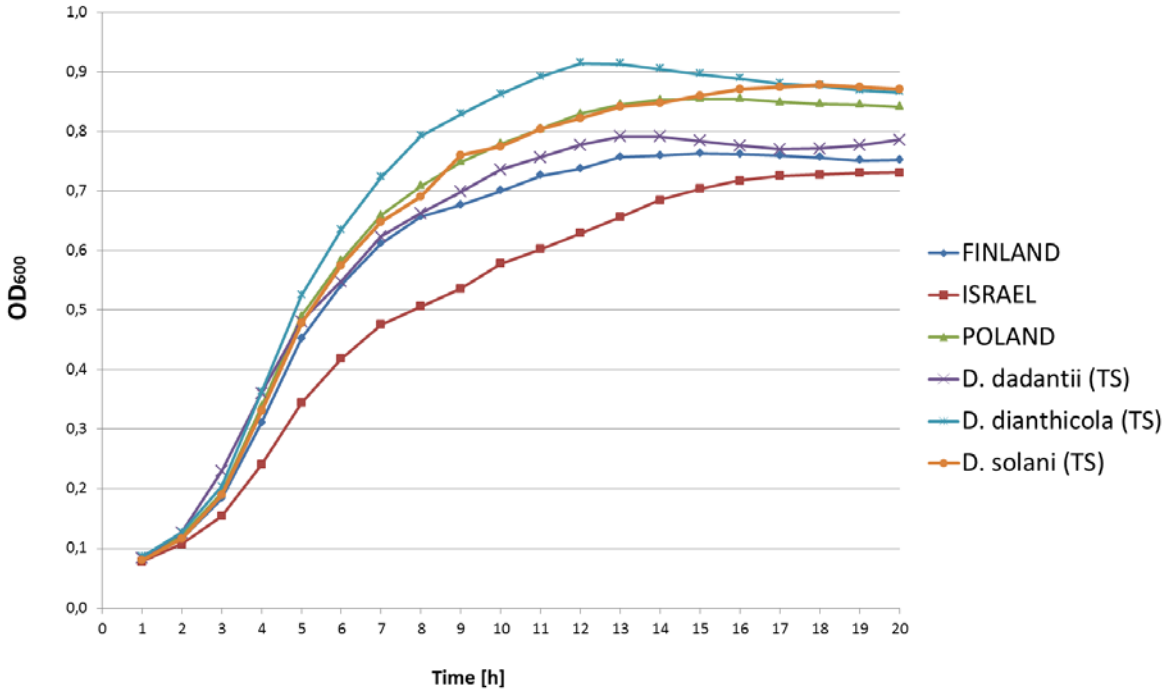

Figure 10. Comparison of growth curves of *D. solani, D. dadantii* and *D. dianthicola* strains at 37 °C.

Interestingly at 37 ºC the Polish strains grow to the stationary phase faster than Finnish and Israeli (after 14.8 h in comparison to 15.6 and 16 h, respectively). However these differences both for the time of reaching the stationary phase as well as the level of the maximum optical density were again not significant (confirmed by one-way ANOVA, $p > 0.05$).

Table 11. Growth of *D. solani* strains at 28 and 37 °C in comparison to *D. dadantii* and *D. dianthicola*. The highest and the lowest values of time after *Dickeya* spp. reach the stationary phase are highlighted in orage and blue (respectively).

Dickeya spp.		Time to achieve maximum density [h]		Maximal optical density	
		28 °C	37 °C	28 °C	37 °C
From Poland	IFB 0099	14	15	0.92	0.86
	IFB 0100	15	13	0.90	0.87
	IFB 0158	13	14	0.84	0.89
	IFB 0167	14	16	0.88	0.77
	IFB 0212	13	16	0.82	0.80
	Average	**13.8**	**14.8**	**0.87**	**0.84**
From Finland	IFB 0231	16	16	0.68	0.85
	IFB 0236	16	13	0.64	0.50
	IFB 0254	15	16	0.81	0.79
	IFB 0261	16	16	0.79	0.85
	IFB 0265	15	16	0.82	0.94
	Average	**15.6**	**15.4**	**0.75**	**0.78**
From Israel	IFB 0124	10	16	0.86	0.71
	IFB 0125	16	16	0.73	0.86
	IFB 0455	15	16	0.87	0.68
	IFB 0456	13	16	0.59	0.51
	IFB 0457	10	16	0.90	0.84
	Average	**12.8**	**16**	**0.79**	**0.72**
***D. solani* IFB 0123**		**12**	**16**	**0.77**	**0.87**
All *D. solani* *		**13.9**	**14.4**	**0.80**	**0.77**
D. dadantii		**12**	**13**	**0.79**	**0.79**
D. dianthicola		**14**	**12**	**0.85**	**0.91**

* All *D. solani* - comprises average value for 16 tested *D. solani* strains.

7.1.1.2 Pectinolytic activity of Dickeya spp. in different temperature conditions

Comparison of pectinolytic activity of D. dadantii, D. dianthicola and D. solani in different temperature conditions

The impact of temperature and species was analyzed by two-way ANOVA analysis of variance and it has been found that there is a combined influence of temperature and species

on pectinolytic activity (F=23.488, p<0.005). As shown in Figure 11, there is no significant difference in pectinolytic activity between *D. dadantii* and *D. solani* at 18 and 28 °C, but there is a difference in pectinolytic activity at 37 °C for these two groups. The ability of *D. dianthicola* strains to produce pectinolytic enzymes is significantly lower than those of *D. dadantii* and *D. solani* at all of the three tested temperatures. What is more, *D. solani* produces the most enzymes at 28 °C, while *D. dadantii* produces them at the same level at both temperatures – 28 and 37 °C.

In conclusion: temperature and species have an impact on pectinolytic enzymes activity level, and the activity of pectinases is the highest at 28 °C for all of the tested species, though the activity of *D. diathicola* strains is significantly lower. All the tested species have an ability to produce pectinolytic enzymes at 18, 28 and 37 °C, although *D. dianthicola* at 37 °C has the lowest (close to zero) pectinolytic activity. *D. solani* IFB 0123 has the highest pectinolytic enzymes activity at both 18 and 28 °C.

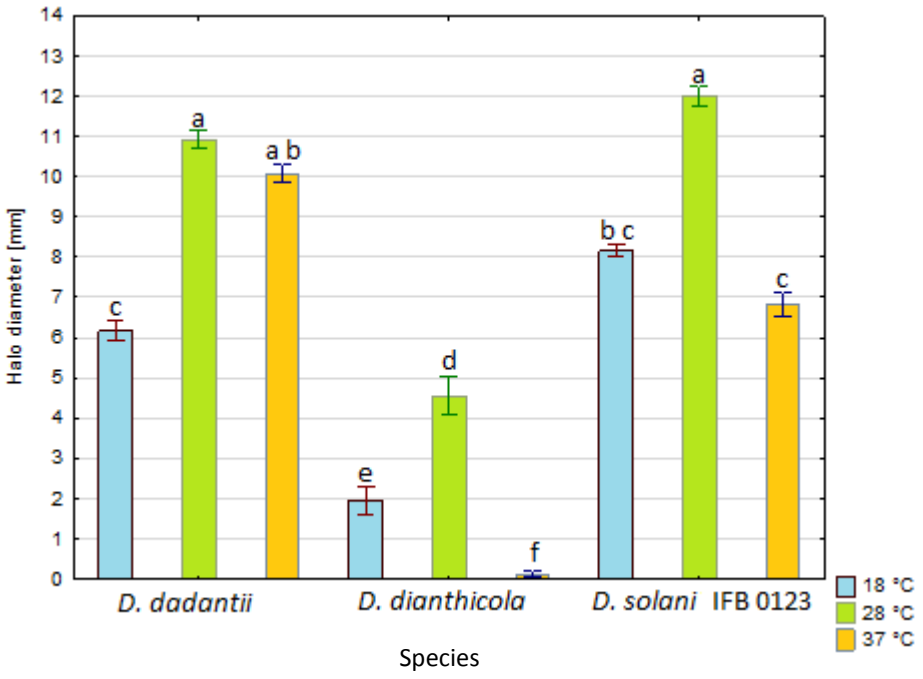

Figure 11. Temperature influence on pectinolytic activity of *D. dadantii, D. dianthicola* and *D. solani* Type Strain. Statistical analysis – two-way ANOVA (F=23.488, p<0.005), followed by post hoc Tukey test. Bar height represents the mean value from 3 repetitions, the whiskers represent the value of the standard error.

Comparison of pectinolytic activity of Dickeya solani strains in different temperature conditions

All of the tested *D. solani* strains produce pectinolytic enzymes at all three tested temperatures. Polish strains seem to be the most similar to each other regarding pectinolytic activity, while the strains from Finland and Israel seem to be more diverse at all temperatures. The highest production of pectinolytic enzymes occurs at 28 °C and it is true for all tested strains. At 18 °C, Finnish strain IFB 0231 and Israeli strain IFB 0456 have a distinguishingly lower level of pectinolytic activity. The results are presented in Figure 12.

Polish strains have a similar pectinolytic activity at all tested temperatures. The activity of those enzymes for Polish strains is the highest at 28 °Cand at 37 °C. Among Finnish strains, one strain – IFB 0231 seems to be different from the other four strains tested. Its pectinolytic activity is the lowest in all tested temperatures among Finnish strains.

At 37 °C, Finnish strains have a lower pectinolytic activity than at 28 °C, but similar to the activity at 18 °C. Israeli strains are the most diverse group. At 18 °C, one strain IFB 0456 has the lowest pectinolytic activity, while other strains are similar to each other. At 28 and 37 °C, there are more differences between strains. IFB 0455 pectinolytic activity is the lowest at both temperatures. At 37 °C, the Israeli strains show the most variance among the group. All Israeli strains have a lower pectinolytic activity at 37 ºC than at 28 °C.

In conclusion: Polish strains produce pectinolytic enzymes at all tested temperatures similarly to each other, while Finnish and Israeli (especially Israeli) strains exhibit variance between strains at different temperatures.

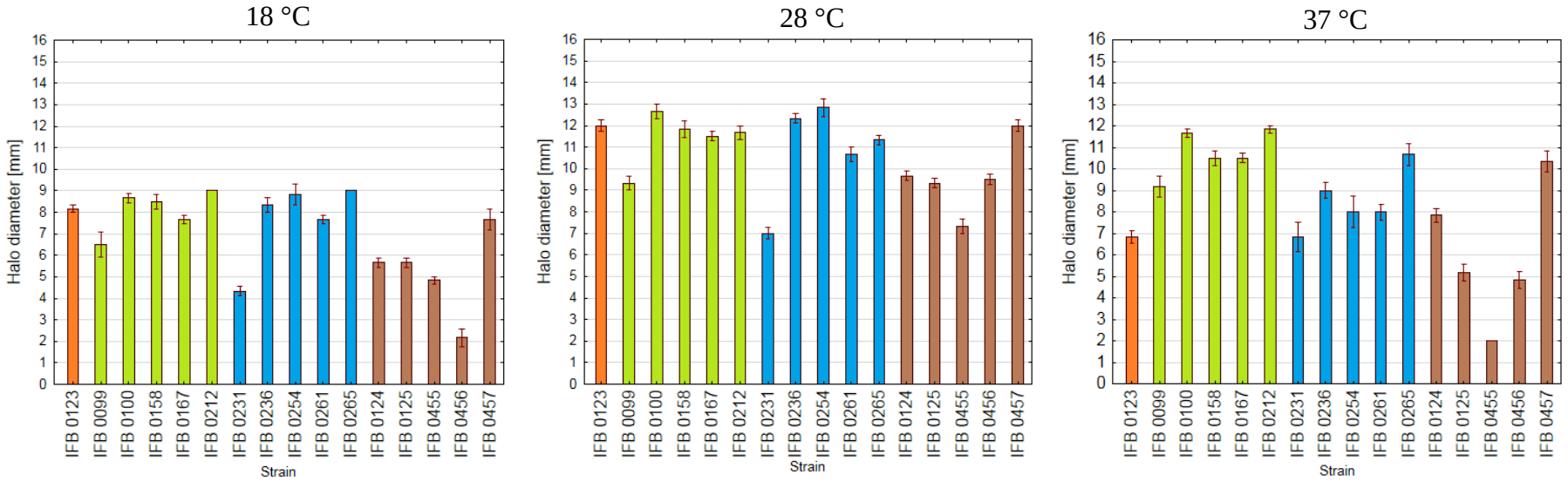

Figure 12. Comparison of *D. solani* strains pectinolytic activity at three different temperatures – 18, 28 and 37 °C. Orange – *D. solani* Type Strain IFB 0123, green – Polish *D. solani* (IFB 0099, IFB 0100, IFB 0158, IFB 0167, IFB 0212), dark blue – Finnish *D. solani* (IFB 0231, IFB 0236, IFB 0254, IFB 0261, IFB 0265), brown – Israeli *D. solani* (IFB 0124, IFB 0125, IFB 0455, IFB 0456, IFB 0457). Bar height represents the mean value from 3 repetitions; the whiskers represent the value of the standard error.

Comparison of pectinolytic activity of D. solani strains of different origin

The impact of temperature and strain origin was analyzed by two-way ANOVA analysis of variance and it was found that there is an influence of temperature and origin on pectinolytic activity ($F=3.927$ $p<0.005$). As seen in the graph (Figure 13) the highest activity of the pectinolytic enzymes is observed at 28 °C for all three groups, while Polish and Finnish strains do not differ significanty from each other at this temperature. Israeli strains, at 28 °C, differ significantly from Polish strains, but not from Finnish. They indicated lower pectinolytic activity than Polish and Finnish strains at all tested temperatures.The pectinolytic activity of Polish strains is the highest at all tested temperatures. The pectinolytic activity of Polish strains is significantly different from Israeli strains at all temperatures but not from Finnish strains. At 18 °C, Polish and Finnish strains produce pectinolytic enzymes at a similar level, but significantly different than Israeli strains whose activity is lower. Finnish and Israeli strains do not differ significantly in the pectinolytic activity at 28 °C.

In conclusion: temperature and strain origin have an impact on pectinolytic enzymes activity level. Polish strains have the highest pectinolytic activity at all tested temperatures, while Israeli strains show the lowest activity at all tested temperatures.

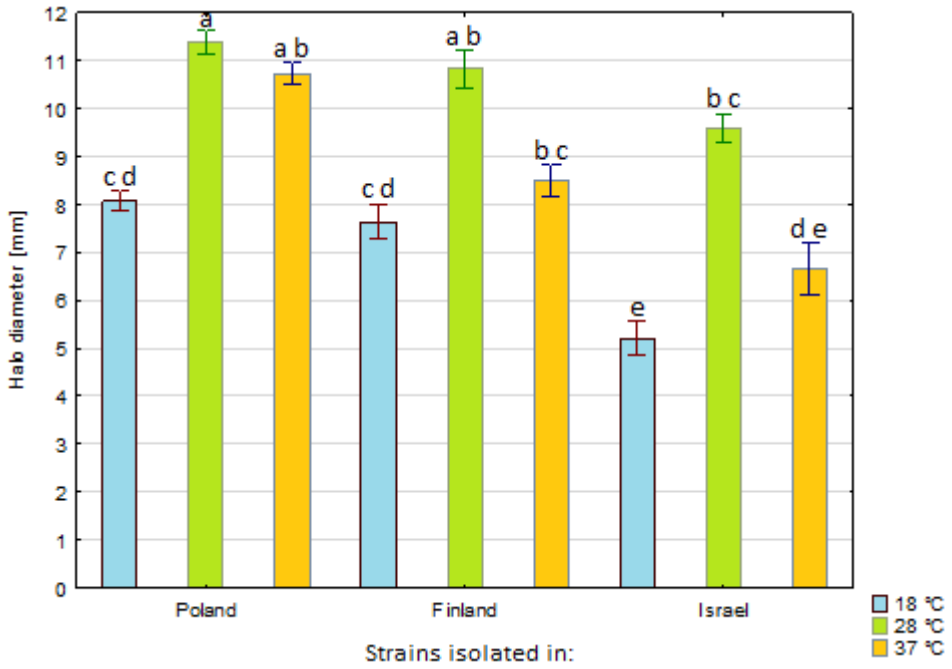

Figure 13. Temperature influence on pectinolytic enzymes production of *D. solani* strains coming from different climatic conditions. Blue – 18 °C, green – 28 °C, orange – 37 °C.Statistical analysis – two-way ANOVA ($F=3.927$ $p<0.005$), followed by post hoc Tukey test. Bar height represents the mean value from 3 repetitions, the whiskers represent the value of the standard error.

7.1.1.3 Cellulolytic activity of Dickeya spp. in different temperature conditions

Cellulolytic activity of D. dadantii, D. dianthicola and D. solani in different temperature conditions

The impact of temperature and species was analyzed by two-way ANOVA analysis of variance and it has been found that there is an influence of temperature and species on cellulolytic activity ($F=13.623$, $p<0.001$). *D. dadantii and D. solani* have the highest cellulolytic activity at higher temperatures such as 28 and 37 °C and they do not differ significantly at those temperatures. They also do not differ significantly at 18 °C in cellulolytic enzymes activity, but this activity is the lowest for the strains of those species at this temperature. The cellulolytic activity of *D. dianthicola* is at similar level at all tested temperatures and at 28 and 37 °C it is significantly lower than those of *D. dadantii* and *D. solani*.

In conclusion: temperature and species have an impact on cellulolytic enzymes activity, and the activity of those enzymes is the highest at 28 and 37 °C for *D. dadantii* and *D. solani*.

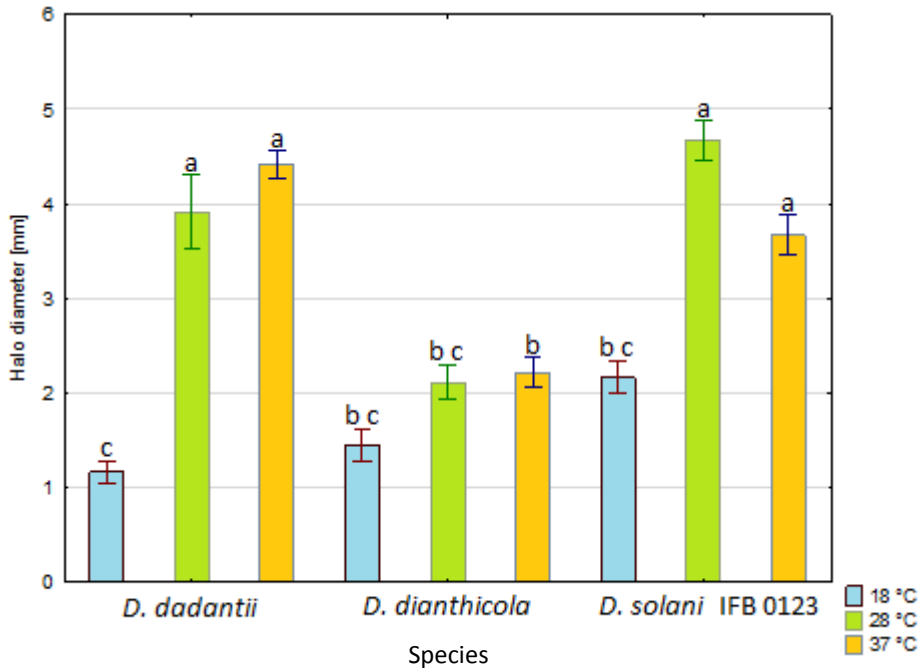

Figure 14. Temperature influence on cellulolytic activity of *D. dadantii, D. dianthicola* and *D. solani* Type Strain. Blue – 18 °C, green – 28 °C, orange – 37 °C. Statistical analysis – two-way ANOVA ($F=13.623$, $p<0.001$), followed by post hoc Tukey test. The height of the bars represents the mean value from 3 repetitions; the whiskers represent the value of the standard error.

Comparison of the cellulolytic activity of D. solani strains in different temperature conditions

All the tested *D. solani* strains produce cellulases. In general, *D. solani* strains cellulolytic activity is the lowest at 18 °C. At 18 °C, all strains from different climatic conditions and the *D. solani* TS have a similar activity level. At 28 °C, the strains vary between each other. Polish strains are most similar to each other at 28 °C. Again one of Finnish strains – IFB 0231 is different than the other four and its cellulolytic activity is the lowest. The cellulolytic activity of the other four strains is similar to Polish strains. The strains from Israel exhibit the opposite, four out of five tested Israeli strains show lower cellulases production than the rest of *D. solani* strains at 28 °C, only one (IFB 0457) shows high cellulases activity level (similar to strains from Poland and Finland). The *D. solani* TS indicates low cellulolytic activity in comparison to strains: Finnish IFB 0231 and four Israeli strains – IFB 0124, IFB 0125, IFB 0455 and IFB 0456. At 37 ºC, Polish strains cellulolytic activity is the most varied. Each strain differs from another. At 37 ºC, two of the Finnish strains (IFB 0231 and IFB 0236) have a lower cellulolytic activity than the other three. At 37 ºC, one Israeli strain (IFB 0457) again has a higher cellulolytic activity than the other four.

In conclusion: *D. solani* strains have the highest cellulolytic activity at 28 °C. Again, Polish strains are the most similar to each other while strains from Finland and Israel are more diverse.

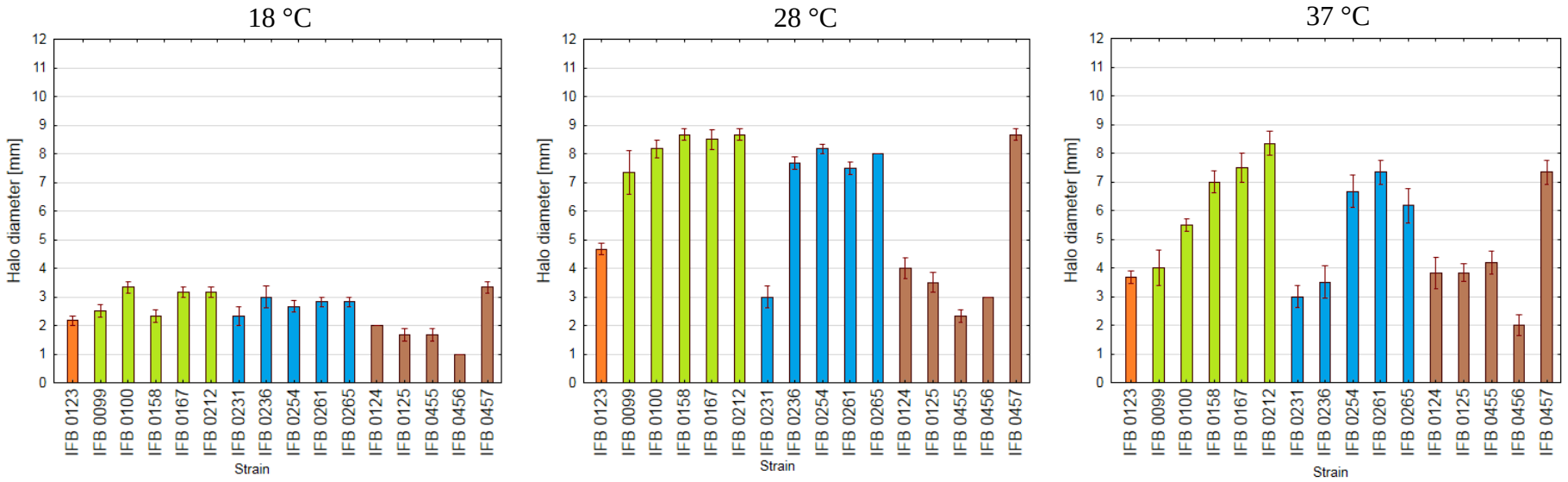

Figure 15. Comparison of *Dickeya* spp. cellulolytic activity at three different temperatures. Orange – *D. solani* Type Strain IFB 0123, green – Polish *D. solani* (IFB 0099, IFB 0100, IFB 0158, IFB 0167, IFB 0212), dark blue – Finnish *D. solani* (IFB 0231, IFB 0236, IFB 0254, IFB 0261, IFB 0265), brown – Israeli *D. solani* (IFB 0124, IFB 0125, IFB 0455, IFB 0456, IFB 0457). Bar height represents the mean value from 3 repetitions; the whiskers represent the value of the standard error.

Comparison of cellulolytic activity of D. solani strains of different origin

The impact of temperature along with the strain origin was analyzed by two-way ANOVA analysis of variance and it has been found that there is an influence of the temperature and origin on cellulolytic activity of the tested groups ($F=6.717$, $p<0.005$). As seen in the graph (Figure 16) the highest amount of the cellulolytic enzymes is produced at 28 °C by Polish strains and it is significantly different from the Finnish and Israeli strains at this temperature. Israeli strains, at 37 °C, differ significantly from Polish strains, but not from Finnish. The amount of enzymes produced by Polish strains at 18 °C is comparable to the amount of enzymes produced by Finnish and Israeli strains at 18 °C. At 18 °C, all of the groups produce the least of cellulolytic enzymes. At 28 and 37 °C, Israeli strains produce similar amount of cellulolytic enzymes.

In conclusion: temperature and strain origin have an impact on cellulolytic enzymes activity level, and the activity of those enzymes is the highest at 28 °C for all of the tested species, though it differs significantly between them. Polish strains have the highest cellulolytic activity at both: 28 and 37 °C. The cellulolytic activity of Finnish and Israeli strains is significantly lower than of Polish ones at 28 °C.

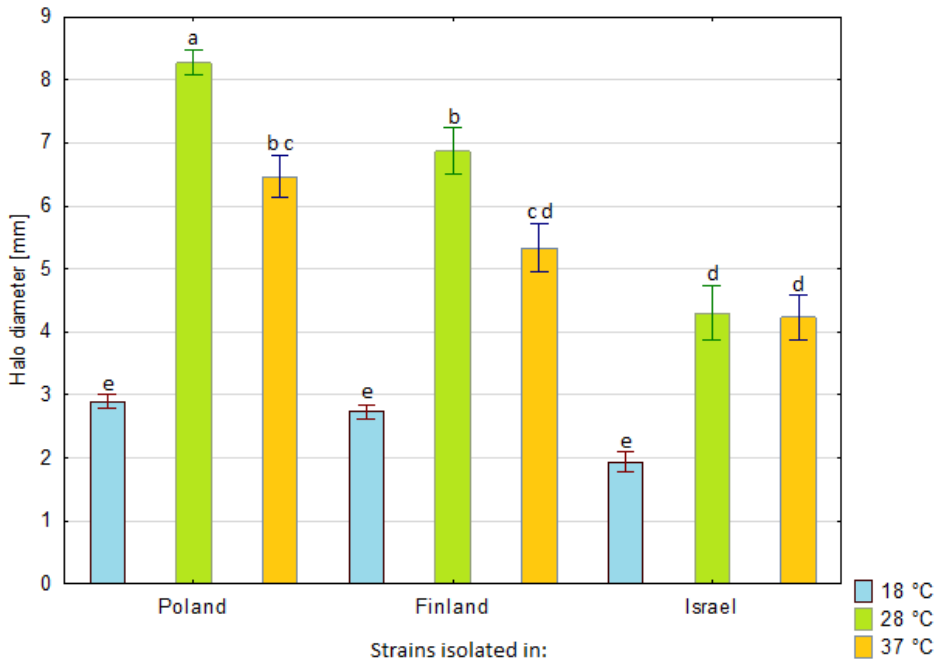

Figure 16. Temperature influence on cellulolytic enzymes production of *D. solani* strains coming from different climatic conditions. Blue – 18 °C, green – 28 °C, orange – 37 °C. Statistical analysis – two-way ANOVA ($F=13.623$, $p<0.001$), followed by post hoc Tukey test.The height of the bars represents the mean value from 3 repetitions, the whiskers represent the value of the standard error.

7.1.1.4 Proteolytic activity of Dickeya spp. in different temperature conditions

Comparison of D. dadantii, D. dianthicola and D. solani proteolytic activity in different temperature conditions

The impact of temperature and species was analyzed by two-way ANOVA analysis of variance and it has been found that there is an influence of temperature and species on proteolytic activity ($F=221.045$, $p<0.001$). As shown in Figure 17, *D. solani* IFB 0123 has a significantly higher proteolytic activity at 18 and 28 °C than *D. dadantii* and *D. dianthicola*. *D. solani* Type strain (IFB 0123) does not have proteolytic activity at 37 °C. The proteolytic activity of *D. dadantii* is similar at both 28 and 37 °C. *D. dianthicola* has no proteolytic activity at both 18 and 37 °C, while at 28 °C it is very low.

In conclusion: temperature and species have an impact on proteolytic enzymes activity level, and the activity of those enzymes is the highest at 28 °C for all of the tested species, though it differs significantly between them. *D. solani* has a higher proteolytic activity than *D. dadantii* and *D. dianthicola* at both 18 and 28 °C.

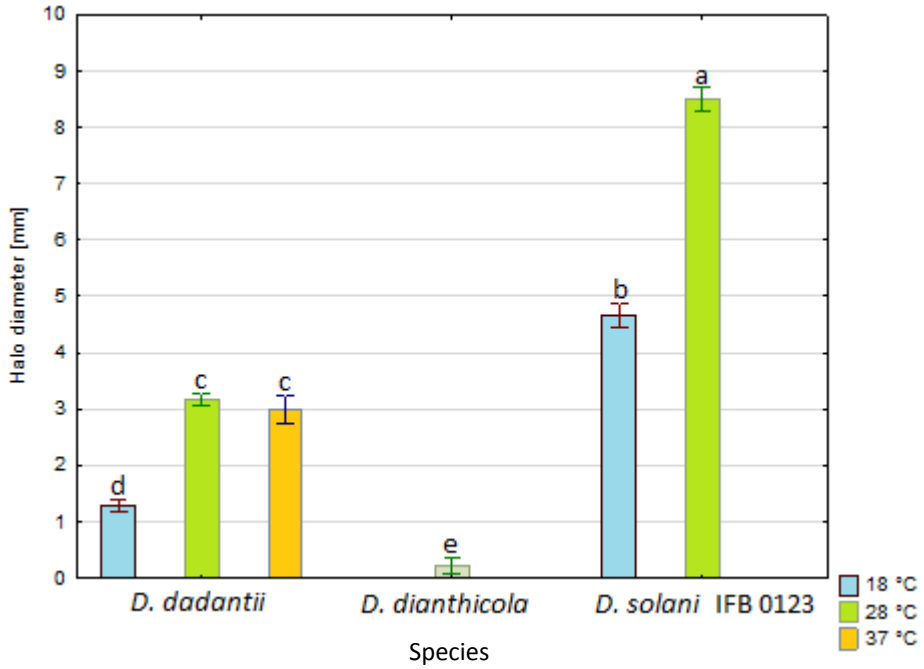

Figure 17. Temperature influence on proteolytic activity of *D. dadantii, D. dianthicola* and *D. solani* Type Strain. Blue – 18 °C, green – 28 °C, orange – 37 °C. Statistical analysis – two-way ANOVA ($F=221.045$, $p<0.001$), followed by post hoc Tukey test. The height of the bars represents the mean value from 3 repetitions, the whiskers represent the value of the standard error.

Comparison of proteolytic activity of D. solani strains in different temperature conditions

All the tested *D. solani* strains have proteolytic activity at 28 °C. The Polish strains again are similar to each other in their proteolytic activity at both 18 and 28 °C. Finnish and Israeli strains are again more diverse at 28 °C than Polish strains. Again, Finnish strain IFB 0231 has the lowest enzymatic activity among Finnish strains at 28 °C. The rest of Finnish strains are similar to Polish strains. Israeli strains are again the most diverse at 28 °C. Three strains (IFB 0124, IFB 0125 and IFB 0455) have a lower proteolytic activity, while two others (IFB 0456 and IFB 0457) have an activity similar to Polish strains at 28 °C. At 18 °C, not all of *D. solani* strains have a proteolytic activity. Two out of 16 tested strains (Finnish strain IFB 0231 and Israeli strain IFB 0125) have no proteolytic activity at 18 °C. One Polish strain (IFB 0158) has a lower proteolytic activity than others. Also at 37 °C, not all strains possess proteolytic activity. Only 6 out of 16 tested strains have proteolytic activity (3 Polish, 1 Finnish and 2 Israeli), but this activity is much lower than at 28 °C for the same strains. What is interesting, *D. solani* ^{TS} (IFB 0123) has the highest proteolytic activity at both 18 and 28 °C.

In conclusion: *D. solani* produces proteolytic enzymes, but this activity is varied among the strains and temperatures.

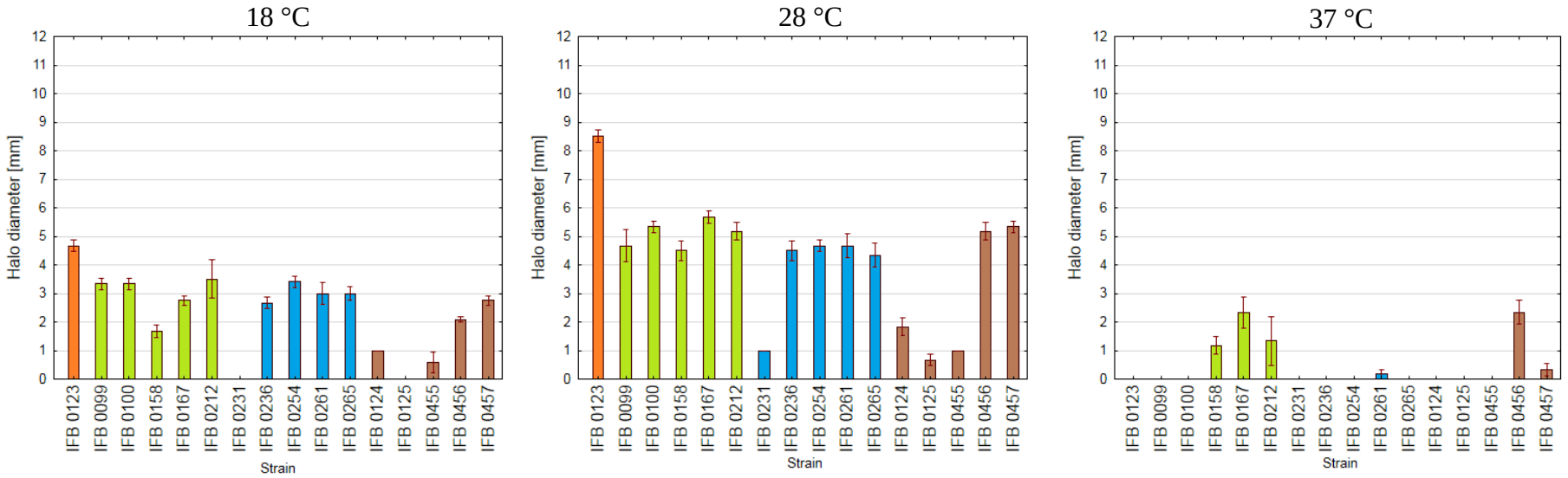

Figure 18. Comparison of *Dickeya* spp. proteolytic activity at three different temperatures. Orange – *D. solani* Type Strain IFB 0123, green – Polish *D. solani* (IFB 0099, IFB 0100, IFB 0158, IFB 0167, IFB 0212), dark blue – Finnish *D. solani* (IFB 0231, IFB 0236, IFB 0254, IFB 0261, IFB 0265), brown – Israeli *D. solani* (IFB 0124, IFB 0125, IFB 0455, IFB 0456, IFB 0457). Bar height represents the mean value from 3 repetitions; the whiskers represent the value of the standard error.

Comparison of proteolytic activity of D. solani strains of different orgin

The impact of temperature and strain origin was analyzed by two-way ANOVA analysis of variance and it has been found that there is an influence of temperature and origin of strain on proteolytic activity ($F=5.422$, $p<0.001$). Polish *D. solani* strains have the highest proteolytic activities at 28 °C and they are significantly higher than Finnish and Israeli strains. At this temperature though, there is no significant difference between Finnish and Israeli strains. Polish strains produce proteases at 18 ºC at a similar level as Finnish and Israeli strains at 28 ºC. What is more, the level of enzymes produced by Polish strains at 18 ºC is also similar to the enzymes amount produced by Finnish strains at the same temperature, but different from Israeli strains.

In conclusion: temperature and species origin have an impact on proteolytic enzymes activity level, and the activity of those enzymes is the highest at 28 °C for all of the tested species, though it differs significantly between them. Again, Polish strains have the highest enzymatic activity at both 18 and 28 °C.

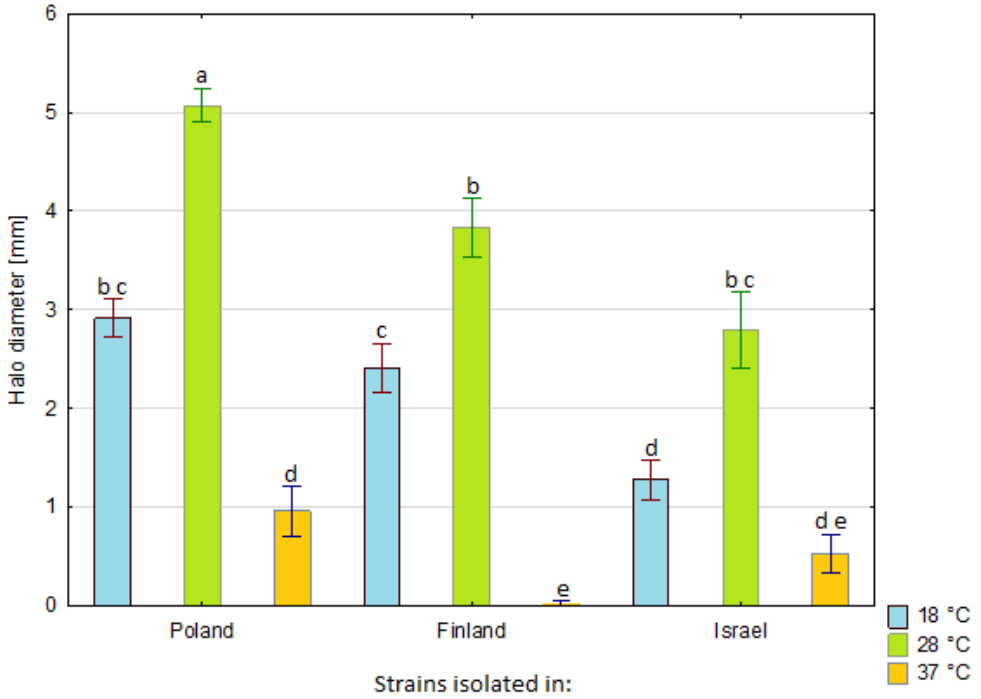

Figure 19. Temperature influence on proteolytic enzymes production of *D. solani* strains coming from different climatic conditions. Blue – 18 °C, green – 28 °C, orange – 37 °C. Statistical analysis – two-way ANOVA ($F=13.623$, $p<0.001$), followed by post hoc Tukey test. Bar height represents the mean value from 3 repetitions; the whiskers represent the value of the standard error.

7.1.1.5 Ability to chelate iron ions by Dickeya spp. in different temperature conditions

Comparison of ability to chelate iron ions by D. dadantii, D. dianthicola and D. solani in different temperature conditions

The impact of temperature and species was analyzed by two-way ANOVA analysis of variance and it has been found that there is an influence of temperature and species on the ability to chelate iron ($F=14.077$, $p<0.001$). As shown in Figure 20, the highest ability to chelate iron ions possesses *D. dadantii* at 37 ºC. At the same time, only *D. dadantii* is able to chelate iron ions at 37 °C. At 28 °C, strains of all of tested species have an ability to chelate iron ions and they do not differ significantly from each other. The same is at 18 °C. Moreover the analysis did not show any significant difference in the ability to chelate iron for *D. dianthicola* and *D. solani*^{TS} at temperatures 18 and 28 °C.

In conclusion: temperature and species have an impact on the ability to chelate iron ions. *D. dadantii* has the best ability to chelate iron ions at 37 °C, while *D. dianthicola* and *D. solani* do not possess this ability at this temperature. At the same time those species do not differ in the ability to chelate iron ions at the other two temperatures (18 and 28 °C).

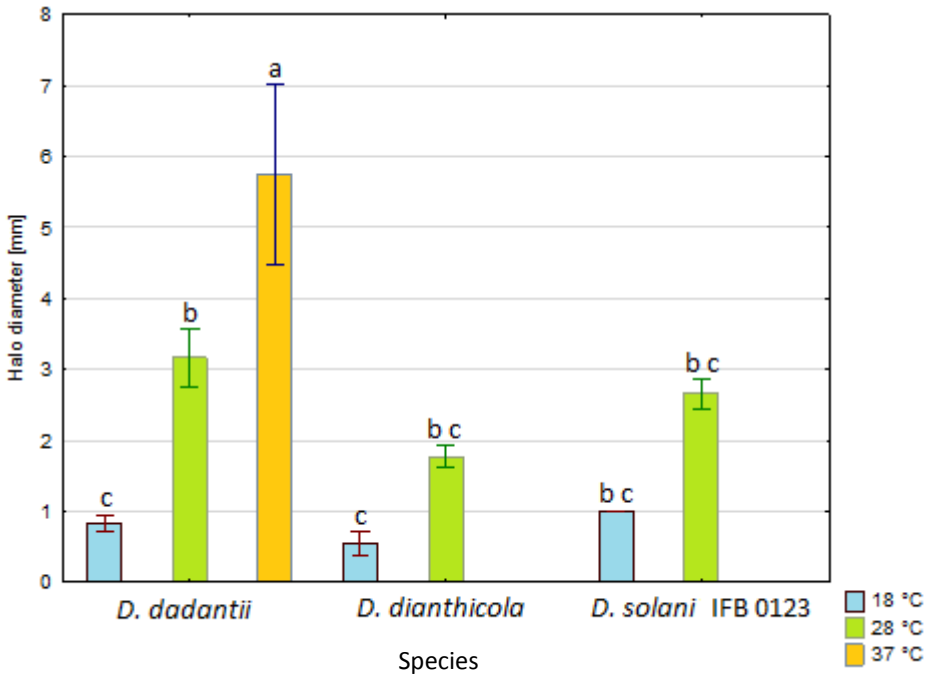

Figure 20. Temperature influence on ability to chelate iron ions of *D. dadantii, D. dianthicola* and *D. solani* Type Strain. Blue – 18 °C, green – 28 °C, orange – 37 °C. Statistical analysis – two-way ANOVA ($F=14.077$, $p<0.001$) followed by post hoc Tukey test. Bar height represents the mean value from 3 repetitions, the whiskers represent the value of the standard error.

Comparison of D. solani strains ability to chelate iron ions in different temperature conditions

All of the tested strains are able to chelate iron ions at 28 °C, but not at 18 or 37 °C. Among 16 tested strains of *D. solani*, two of them do not chelate iron ions at 18 °C (Polish strain IFB 0167 and Israeli strain IFB 0125) and three of them at 37 °C (including Type Strain – IFB 0123, Finnish strain IFB 0236 and Israeli strain IFB 0456). From Finnish *D. solani* group one strain (IFB 0236) has a better ability to chelate iron at 18 and 28 °C, but does not chelate iron ions at 37 °C. The rest of Finnish strains chelate iron ions at the same level at both temperatures 28 and 37 °C. Type Strain of *D. solani* exhibits a similar ability to chelate iron ions at 18 and 28 °C as Polish and Finnish strains. Polish and Finnish strains (besides Finnish strain IFB 0236) are similar to each other in the ability to chelate iron ions at both temperatures: 18 and 28 ºC. Israeli strains are the most varied at 28 ºC.

In conclusion: *D. solani* strains are able to chelate iron ions best at 28 °C and strains from Poland and Finland are similar to each other, while strains from Israel present higher viariability.

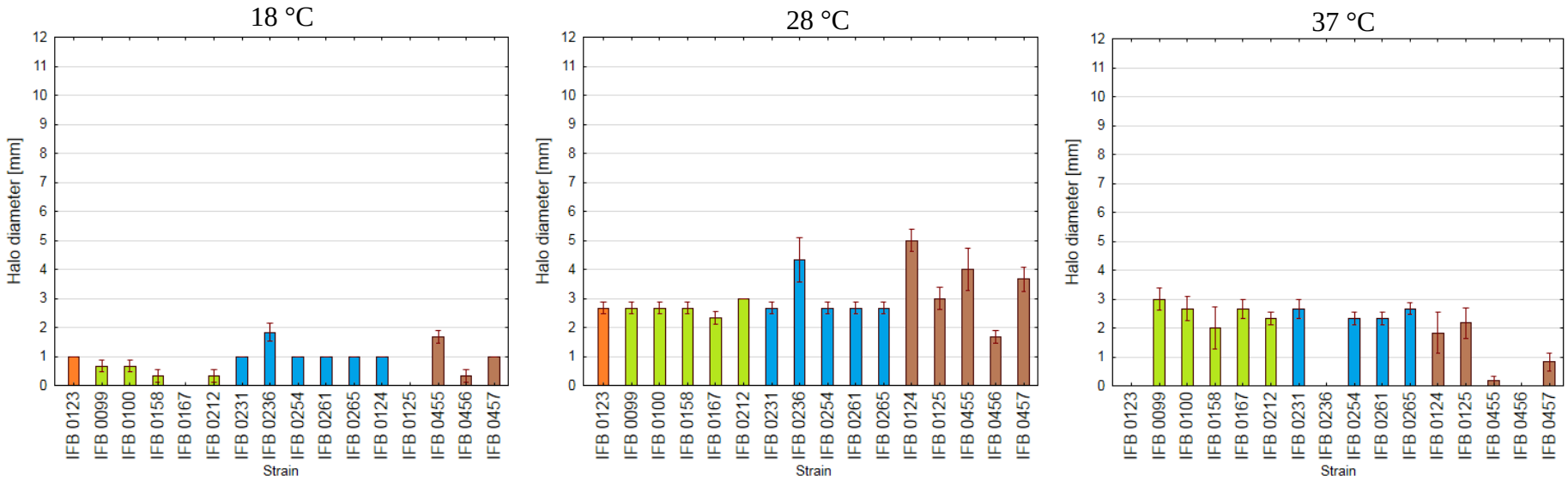

Figure 21. Comparison of *Dickeya* spp. iron chelating ability at three different temperatures. Orange – *D. solani* Type Strain IFB 0123, green – Polish *D. solani* (IFB 0099, IFB 0100, IFB 0158, IFB 0167, IFB 0212), dark blue – Finnish *D. solani* (IFB 0231, IFB 0236, IFB 0254, IFB 0261, IFB 0265), brown – Israeli *D. solani* (IFB 0124, IFB 0125, IFB 0455, IFB 0456, IFB 0457). Bar height represents the mean value from 3 repetitions; the whiskers represent the value of the standard error.

Comparison of ability to chelate iron ions by D. solani strains of different origin

The impact of temperature and strain origin was analyzed by two-way ANOVA (and it has been found that there is an influence of temperature and origin of strain on the ability to chelate iron ions by *D. solani* groups originating from different climats ($F=5.422$, $p<0.001$). As shown in Figure 22, Israeli strains have a higher ability to chelate iron ions at 28 ºC than Finnish and Polish. The activity at 28 °C differs significantly between Israeli and Polish strains, but not between Israeli and Finnish strains. Polish and Finnish strains have a similar ability to chelate iron ions at both temperatures 28 and 37 °C, and at 37 °C it is a higher activity than the activity of Israeli strains.

In conclusion: there is an impact of temperature and origin on the ability to chelate iron ions by *D. solani*. Israeli strains have the highest ability to chelate iron ions at 28 °C, but much lower at 18 and 37 °C. Polish and Finnish strains have a similar ability to chelate iron ions at 28 and 37 °C.

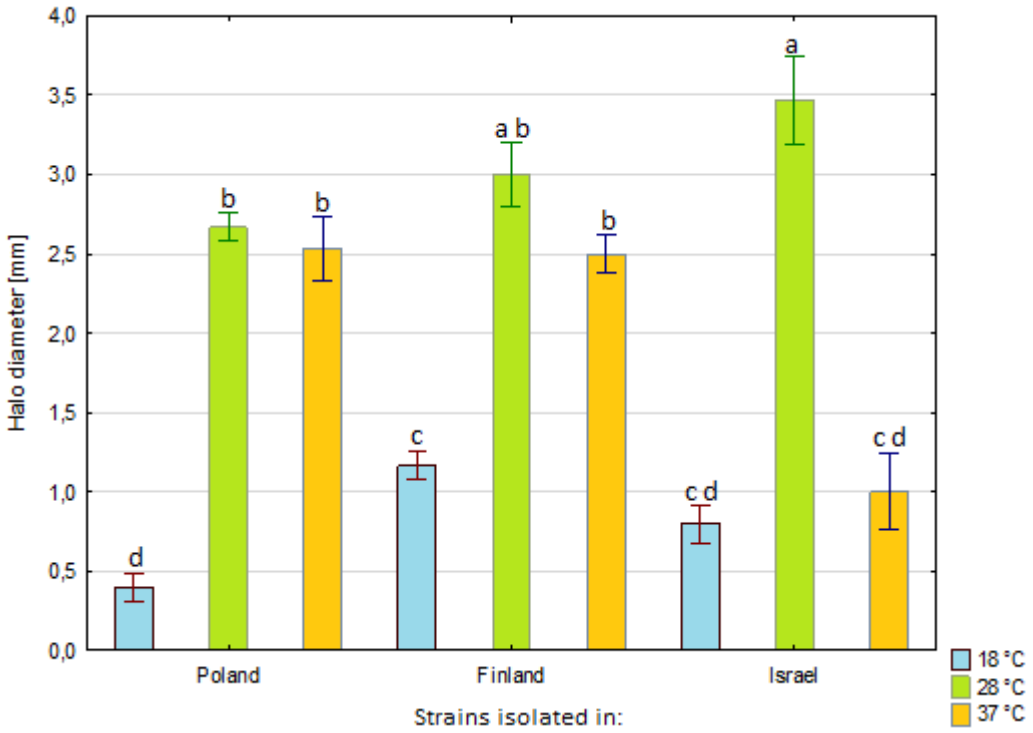

Figure 22. Temperature influence on ability to chelate iron ions of *D. solani* strains coming from different climatic conditions.Blue – 18 °C, green – 28 °C, orange – 37 °C. Statistical analysis – two-way ANOVA ($F=13.623$, $p<0.001$), followed by post hoc Tukey test. Bar height represents the mean value from 3 repetitions, the whiskers represent the value of the standard error.

7.1.1.6 Temperature influence on D. solani strains motility

Two kinds of motility were studied. Swimming and swarming motility are the types of movement typical of bacteria with flagella and they play a role in surface colonization. Both of the traits were tested in two kinds of media with a lowered amount of agar (Methods 6.3.5).

Comparison of swimming ability of D. dadantii, D. dianthicola and D. solani in different temperature conditions

The statistical analysis of temperature and species influence on swimming ability did not confirm any significance, so there is no joint impact of temperature and species on swimming motility ($F=1.11$, $p> 0.001$). But the analysis has shown a significant impact of temperature itself on the swimming motility of the tested strains. For example, the colony diameter at 18, 28 and 37 ºC for *D. solani* was 6, 12 and 5 mm, respectively. All strains indicate the best swimming ability at 28 °C. There is a great difference between the colonized area by the tested groups at 18 and 28 °C. The swimming ability of *D. dadantii* is similar at 28 and 37 °C. *D. dianthicola* and *D. solani* have a better ability to swim at 28 ºC than at 18 or 37 ºC. **In conclusion:** there is no joint impact of temperature and species on the swiming ability of the tested species. But there is a significant influence of the temperature. All tested species have the best swimming ability at 28 °C.

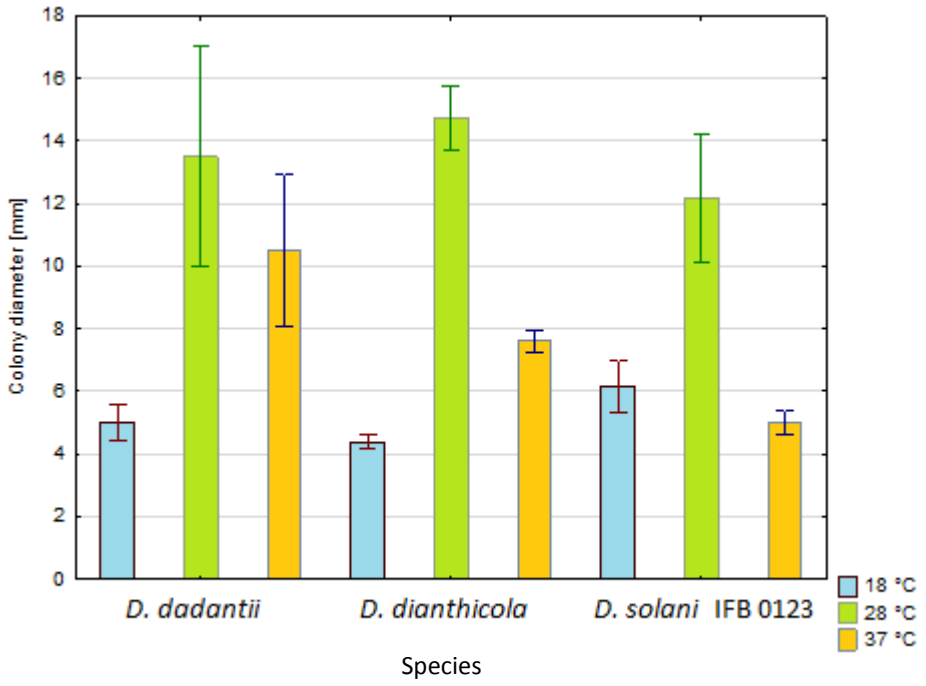

Figure 23. Temperature influence on swimming motility of *D. dadantii*, *D. dianthicola* and *D. solani* Type Strain. Blue – 18 °C, green – 28 °C, orange – 37 °C. Bar height represents the mean value from 3 repetitions, the whiskers represent the value of the standard error.

Comparison of swimming ability of D.solani strains in different temperature conditions

Fourteen out of 16 tested *D. solani* strains indicate the highest swimming ability at 28 ºC. Two other strains (both from Israel, IFB 0124 and IFB 0125) indicated a very low swimming ability at all tested temperatures. The ability to move is low and similar for all tested strains at both temperatures 18 and 37 ºC, but the Israeli strains seem to move less than Polish or Finnish. Polish and Finnish strains are also similar to each other at 28 ºC and they have a better swimming ability than strains from Israel.

In conclusion: Polish and Finnish strains have the best swimming ability among the tested strains at 28 ºC.

Comparison of swimming ability of D. solani strains in different temperature conditions

The impact of temperature and the origin of *D. solani* strains was analyzed by two-way ANOVA. The result is that there is an impact of both traits on the swimming ability of those groups ($F=24.62$, $p<0.001$). In the graph (Figure 25) it is clearly presented that Polish and Finish strains have a better ability to swim at 28 °C than Israeli strains, and the difference is significant. What is more, there is no significant difference between the ability to move at 18 and 37 °C for both groups – strains from Poland and from Finland. Israeli strains have the lowest ability of swimming at every tested temperature and not only do they differ significantly at 28 °C, but also at 18 and 37 °C.

In conclusion: there is an impact of temperature and strain origin on the swimming ability of *D. solani*. Strains originating from Poland and Finland have a greater swimming motility than Israeli strains at 28 ºC. At 18 and 37 ºC all strains have a similar swimming ability.

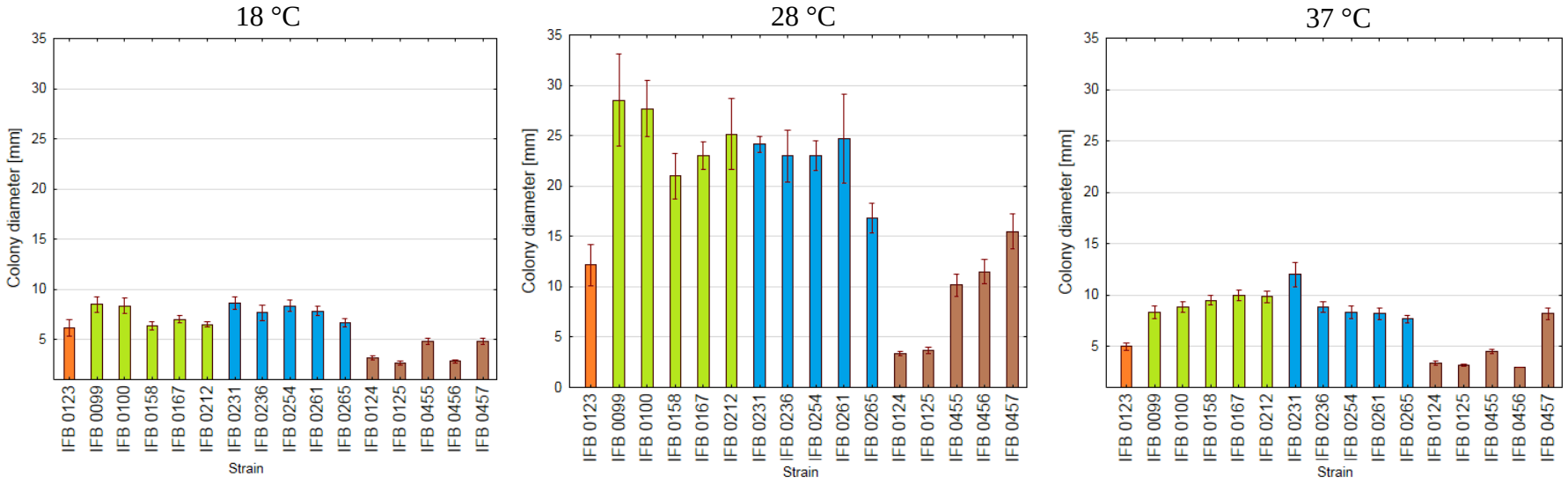

Figure 24. Comparison of *Dickeya* spp. swimming ability at three different temperatures. Orange – *D. solani* Type Strain IFB 0123, green – Polish *D. solani* (IFB 0099, IFB 0100, IFB 0158, IFB 0167, IFB 0212), dark blue – Finnish *D. solani* (IFB 0231, IFB 0236, IFB 0254, IFB 0261, IFB 0265), brown – Israeli *D. solani* (IFB 0124, IFB 0125, IFB 0455, IFB 0456, IFB 0457). Bar height represents the mean value from 3 repetitions; the whiskers represent the value of the standard error.

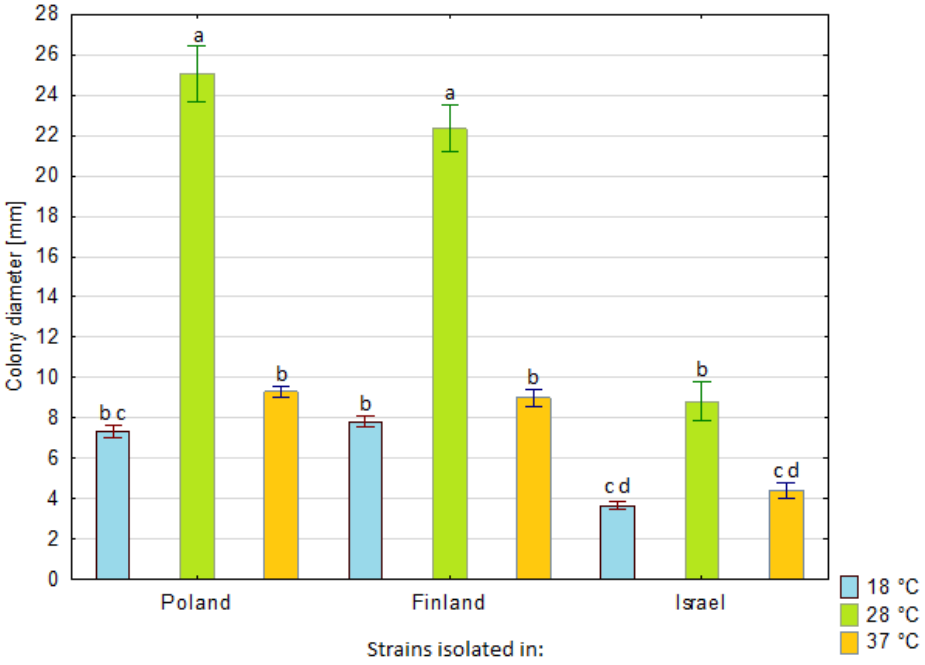

Figure 25. Temperature influence on swimming motility of *D. solani* strains coming from different climatic conditions. Blue – 18 °C, green – 28 °C, orange – 37 °C. Statistical analysis – two-way ANOVA ($F=24.62$, $p<0.001$), followed by post hoc Tukey test. Bar height represents the mean value from 3 repetitions, the whiskers represent the value of the standard error.

Comparison of swarming ability of D. dadantii, D. dianthicola and D. solani in different temperature conditions

The statistical analysis of temperature and species influence on swimming ability did not confirm any significance so there is no joint impact of temperature and species on swimming motility($F=1.74$, $p> 0.001$). But the analysis revealed the impact of the species. As shown in the graph (Figure 26), *D. solani* swarms better than *D. dadantii* and *D. dianthicola* at all tested temperatures, and swarming movement for *D. solani* is similar at all tested temperatures. *D. dadantii* move similarly to *D. dianthicola* at all tested temperatures and there is no difference in surface colonization between temperatures for those species.

In conclusion: there is no joint impact of the temperature and species, but the only significance is observed for species. *D. solani* has a better swarming ability than *D. dadantii* and *D. dianthicola*.

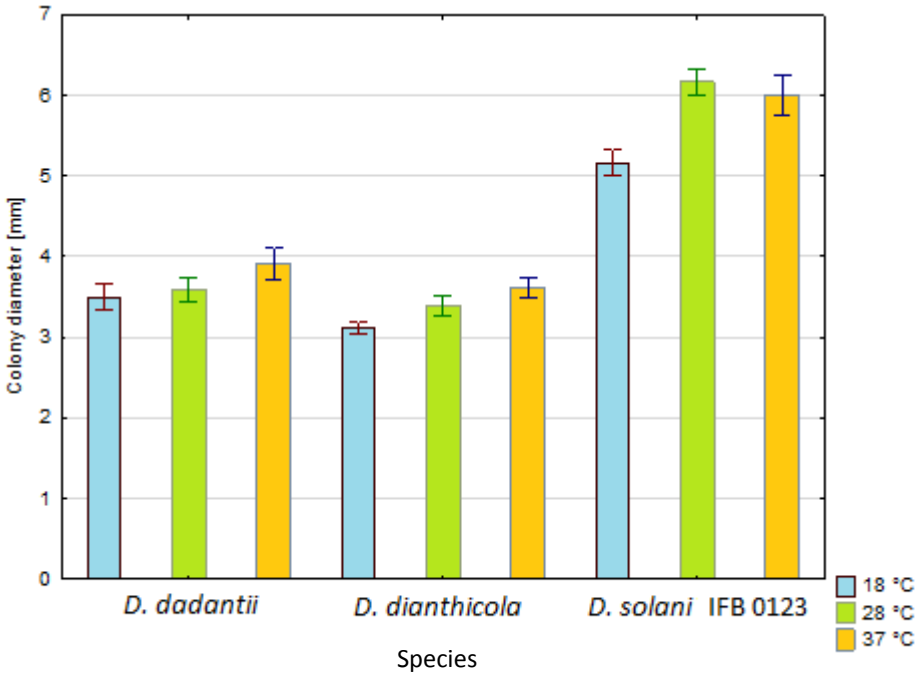

Figure 26. Temperature influence on swarming motility of *D. dadantii*, *D. dianthicola* and *D. solani* Type Strain. Blue – 18 °C, green – 28 °C, orange – 37 °C. Bar height represents the mean value from 3 repetitions, the whiskers represent the value of the standard error.

Comparison of swarming ability of D. solani strains in different temperature conditions

When looking closely at the swarming ability of all tested strains, it is not possible to distinguish big differences between strains, exluding *D. solani*^{TS} (IFB 0123), which seems to have a better ability to swarm at all tested temperatures. One strain of *D. solani* from Finland (IFB 0236) colonizes the surface better than other *D. solani* strains (similarly to Type Strain) at 18 °C but not at two higher temperatures. All tested *D. solani* strains (besides the Type Strain IFB 0123) are similar to each other in their swarming motility, regardless of the temperature.

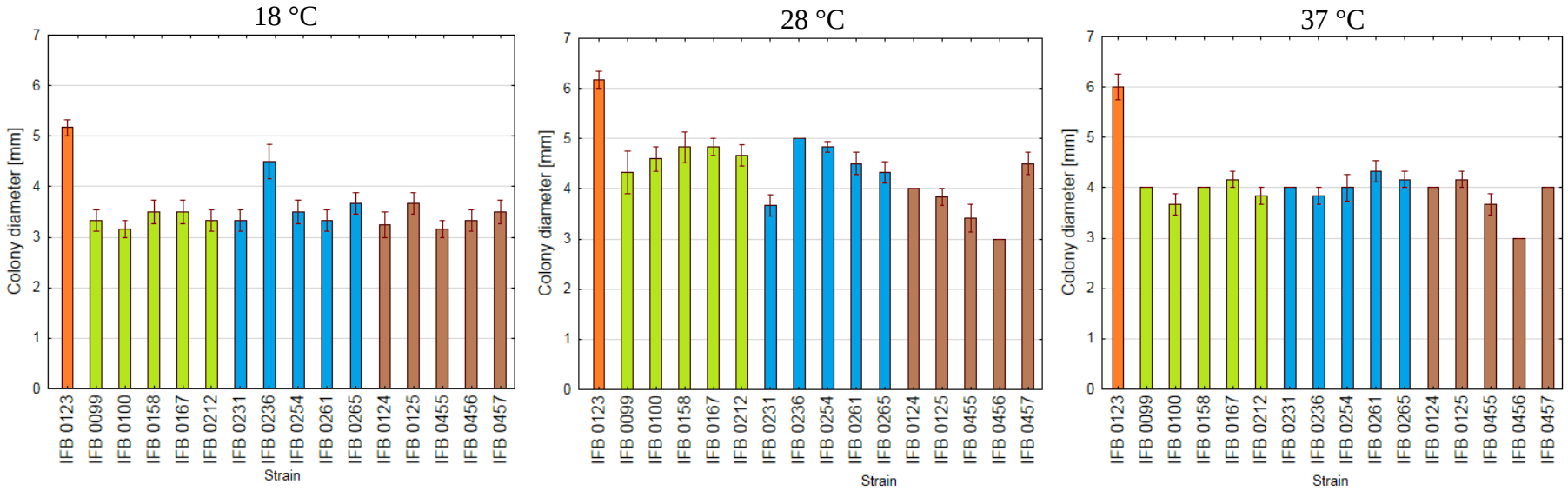

Figure 27. Comparison of *Dickeya* spp. swarming ability at three different temperatures. Orange – *D. solani* Type Strain IFB 0123, green – Polish *D. solani* (IFB 0099, IFB 0100, IFB 0158, IFB 0167, IFB 0212), dark blue – Finnish *D. solani* (IFB 0231, IFB 0236, IFB 0254, IFB 0261, IFB 0265), brown – Israeli *D. solani* (IFB 0124, IFB 0125, IFB 0455, IFB 0456, IFB 0457). Bar height represents the mean value from 3 repetitions; the whiskers represent the value of the standard error.

Comparison of swarming ability of D. solani strains from different origin

The statistical analysis of the impact of temperature and strain origin revealed that there is a joint impact of temperature and strain origin on the swarming ability of *D. solani* strains ($F=5.62$, $p<0.001$). There, significant differences are observed even though the values of colonies diameter are not highly diversified. The Polish and Finnish strains swarm similarly to each other at 28 °C, but significantly differently than Israeli strains at this temperature. The differences are not significant among strains at 18 and 37 °C.

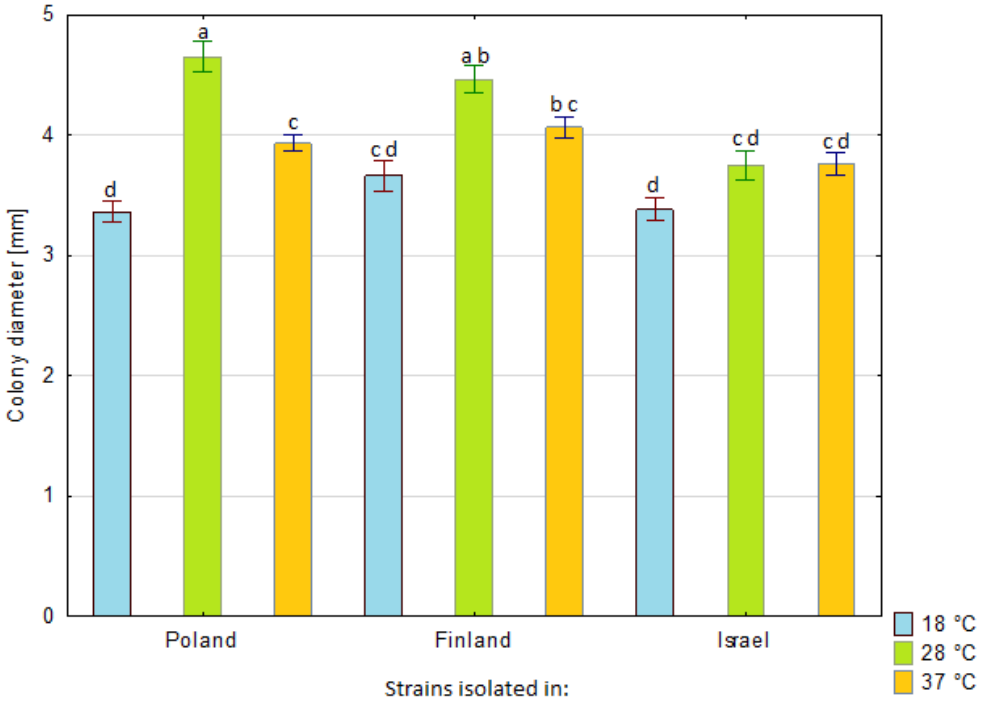

Figure 28. Temperature influence on the swimming motility of *D. solani* strains coming from different climatic conditions. Blue – 18 °C, green – 28 °C, orange – 37 °C. Statistical analysis – two-way ANOVA ($F=13.623$, $p<0.001$), followed by post hoc Tukey test.Bar height represents the mean value from 3 repetitions; the whiskers represent the value of the standard error.

7.1.2 Analyses of the total pectate lyase activity of *D. solani* strains from different climatic conditions

To establish the total pectate lyases activity quanitatively by spectrophotometric method one temperature was selected. It was chosen on the basis of the results obtained in the plate tests. As *D. solani* has the highest pectinases activity at 28 ºC, this temperature was selected to perform quantitative measurements. The total pectate lyases activity was tested under non-induced (in M63 Y minimal medium, Materials 5.5.4) as well as in induced conditions (M63

Y supplemented with 0.2 % PGA, Materials 5.5.5). The total pectate lyases activity of *D. solani* strains originating from different climatic conditions was compared to *D. solani* Type Strain IFB 0123.

Groups of *D. solani* strains were analyzed statistically by two-way ANOVA (for the impact of conditions applied and the origin of the strain). The analysis revealed that there is an impact of both traits on pectate lyases activity ($F=14.07$, $p<0.001$). There is a significant difference between non-induced and induced conditions for all tested *D. solani* groups. It is shown in the graph (Figure 29) that all of the tested groups have a higher pectate lyases activity in induced conditions than in non-induced conditions, which is expressed in the value of induction ratio (Table 12). Polish strains have the highest pectate lyases activities in both non-induced and induced conditions. Because Polish strains have a significantly higher activity in non-induced conditions, the induction ratio for those strains is the lowest (even though they also have the highest pectate lyases activity in induced conditions, Figure 29, Table 12). Strains from Poland, Finland and Israel differ significantly in pectate lyases activity in induced conditions. Polish strains have the highest total pectate lyases activity while Israeli strains have the lowest.

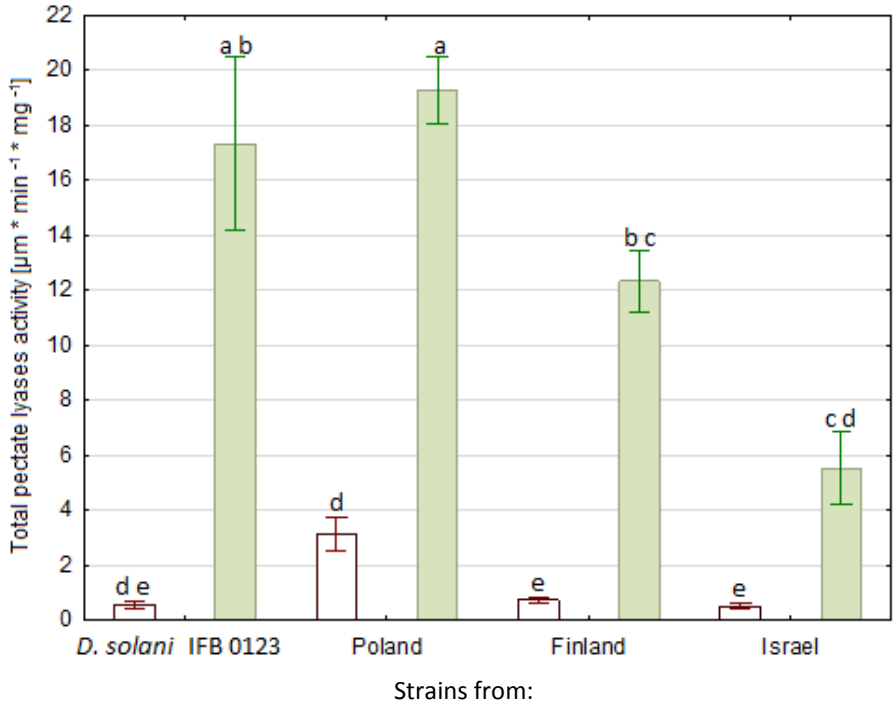

Figure 29. Average total pectate lyases activity of *D. solani* groups in comparison to type strain, induced and non-induced conditions. White – non-induced conditions, grey – induced conditions. The statistical analysis was two-way ANOVA ($F=14.07$, $p<0.001$), followed by post hoc Tuckey test. Bar height represents the mean value from 3 repetitions, the whiskers represent the value of standard error.

Table 12. Induction ratio of the total pectate lysases activity for *D. solani* strains from different climatic conditions in comparison to the Type Strain. The highest values are highlighted in orange.

***D. solani* strains**	**Average specific pectinolytic activity for non-induced conditions**	**Average specific pectinolytic activity for induced conditions**	**Induction ratio**
Poland (average for 5 strains)	3.11	19.28	6.20
Finland (average for 5 strains)	0.74	12.32	16.54
Israel (average for 5 strains)	0.51	5.52	10.91
Type Strain IFB 0123	0.56	17.31	30.76

When taking a closer look at strains in both tested conditions separately, it is observed that strains among groups differ meaningfully (Figure 30). *D. solani* strains from different climatic conditions were compared to the Type Strain (IFB 0123). In non-induced conditions two of Polish *D. solani* strains (IFB 0099 and IFB 0100) have approx. 3 times higher pectate lyases activity than other tested strains. In induced conditions Polish *D. solani* strains have on average the highest pectate lyases activity (one strain only has 2 times lower activity among the group, IFB 0158). The Finnish strains in induced conditions are more diverse, two strains have high (and comparable activity to Polish *D. solani* strains, IFB 0236 and IFB 0261), two of them have intermediate activity (IFB 0254 and IFB 0265) and one has very low activity in induced conditions (IFB 0231). But, what is very interesting, four out of five Israeli strains have very low pectate lyases activity in induced conditions (IFB 0124, IFB 0125, IFB 0455 and IFB 0456) and only one strain (IFB 0457) has an activity on the level comparable to Polish *D. solani* strains.

In conclusion: Polish strains have the highest total pectate lyases activity in both tested conditions.

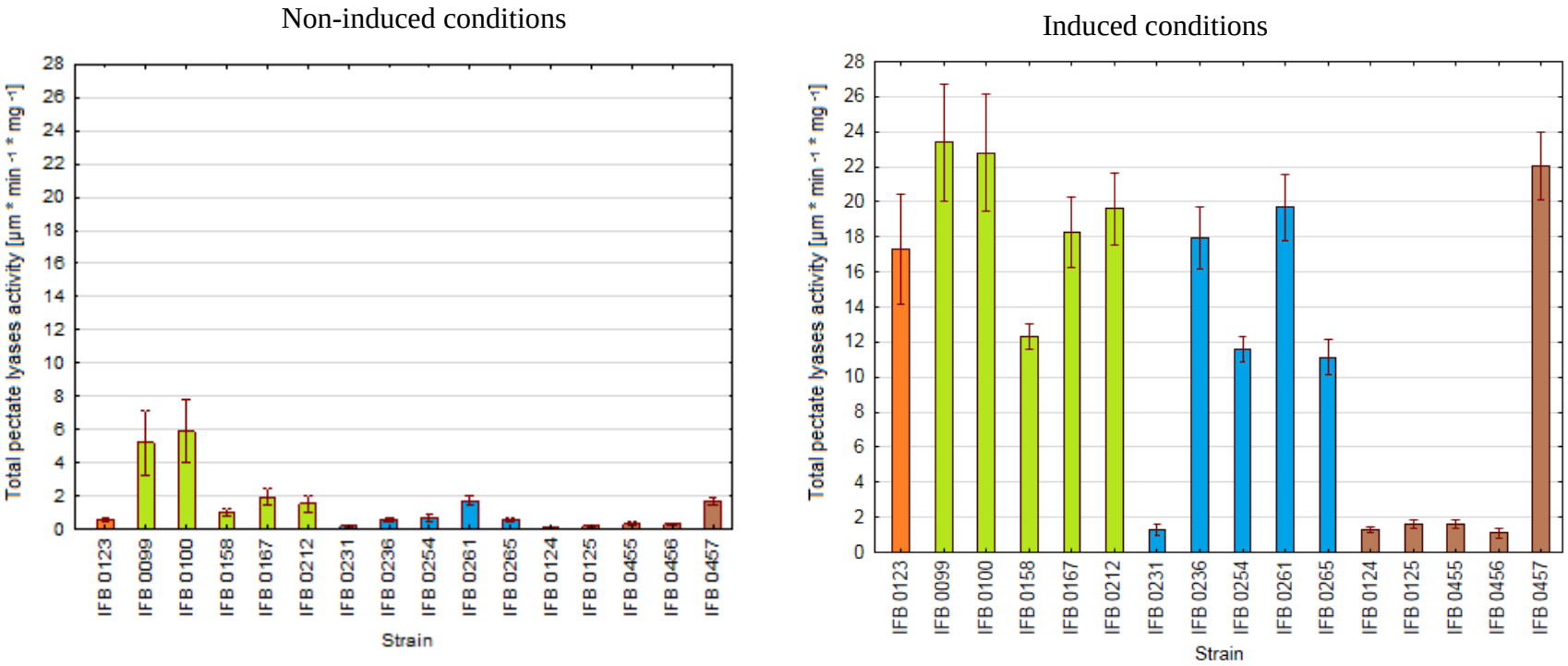

Figure 30. Average total pectate lyases activity of *D. solani* strains in comparison to Type Strain (IFB 0123). Orange – *D. solani* Type Strain IFB 0123, green – Polish *D. solani* (IFB 0099, IFB 0100, IFB 0158, IFB 0167, IFB 0212), dark blue – Finnish *D. solani* (IFB 0231, IFB 0236, IFB 0254, IFB 0261, IFB 0265), brown – Israeli *D. solani* (IFB 0124, IFB 0125, IFB 0455, IFB 0456, IFB 0457). Bar height represents the mean value from 3 repetitions; the whiskers represent the value of the standard error.

7.1.3 Comparison of the maceration ability of *D. solani* strains from different climatic conditions

To test the maceration ability of the *D. solani* strains originating from different climatic conditions, the temperature of 28 °C and 3 different levels of inoculum (10^5, 10^6 and 10^7 CFU/ml) were chosen. The groups of strains were analyzed statistically by two-way ANOVA to establish if there is a joint impact of inoculums level and species origin on potato tissue maceration ability. The groups of strains originating from different climatic conditions were tested in comparison to the *D. solani* Type Strain IFB 0123. Statistical analysis did not show the joint impact of inoculums level and species origin ($F=1.98$, $p>0.001$), but differences among the tested groups were observed. Strains from Poland have the best ability to macerate potato tissue at all tested inoculums levels (Figure 31). Israeli strains have the lowest ability to macerate potato.

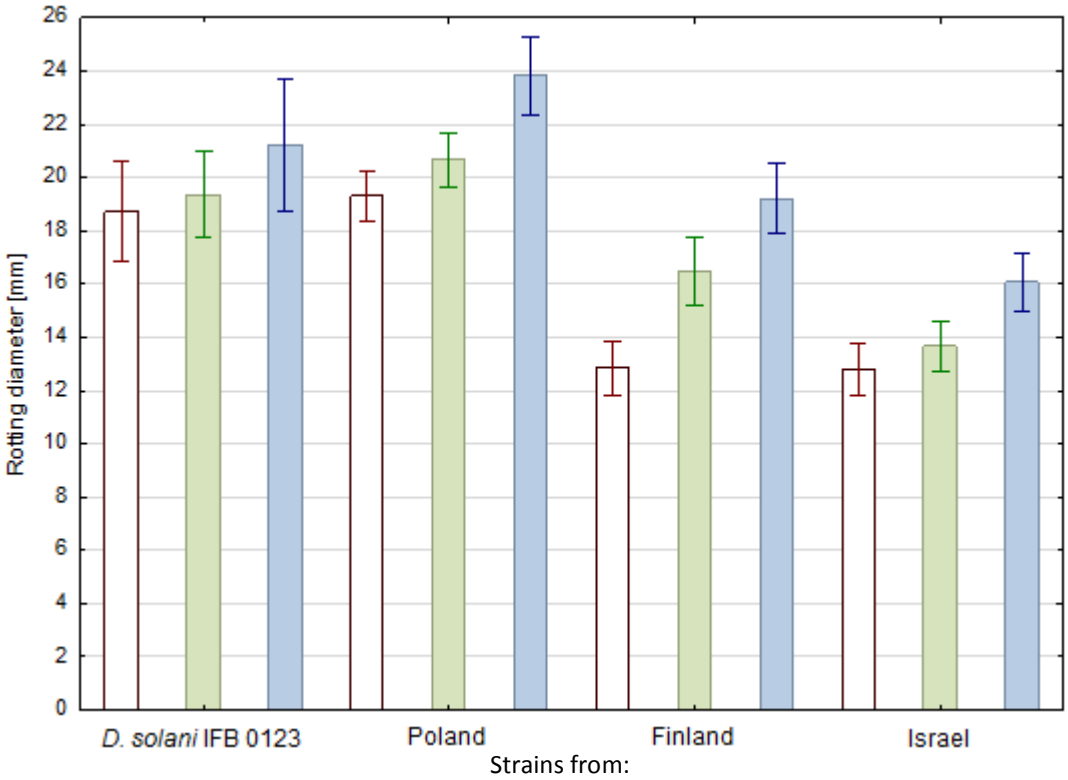

Figure 31. Maceration ability of *D. solani* strains from different climatic conditions at the different inoculum levels. White– inoculum 10^5, green – inoculum 10^6 and blue – inoculum 10^7 CFU/ml. Bar height represents the mean value from 2 repetitions, the whiskers represent the value of standard error.

Differences in the ability to macerate potato tissue between groups were observed at 10^6 and 10^7CFU/ml inoculum level. Inoculum of 10^5CFU/ml could not distinguish the difference between groups of Finnish and Israeli strains. Strains from Finland generally have a moderate ability to macerate potato, and Israeli strains macerate potato tissue less efficiently than other strains of *D. solani* at all tested inoculum levels.

In conclusion: Polish strains of *D. solani* have a better potato maceration ability than the strains from other tested groups.

In Figure 32 is presented the ability to macerate potato tubers for single strains. Polish strains again seem to be the most homogenous group and all of the strains have similar ability to macerate potato tissue at all tested inoculums levels. Finnish and Israeli strains are more diverse than Polish strains. One of the Finnish strains (IFB 0231) has a much lower ability to macerate potato than other Finish strains at all tested levels of inoculum.Two Israeli strains (IFB 0125 and IFB 0455) exhibit a lower ability of potato maceration at inoculums levels 10^6 and 10^7 CFU/ml. In general, Israeli strains have a lower ability to macerate potato than Polish strains.

In conclusion: Polish strains have a better ability to macerate potato tissue at all tested inoculums levels than Finnish or Israeli strains.

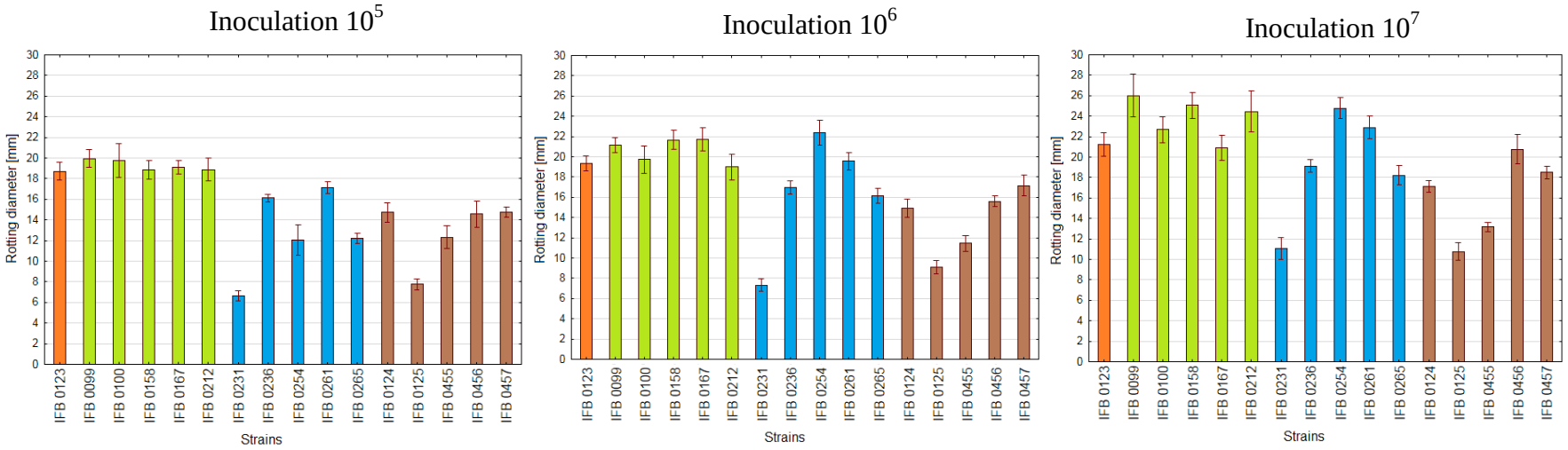

Figure 32. Maceration ability of *D. solani* strains from different climatic conditions at different inoculum levels. Orange – *D. solani* Type Strain IFB 0123, green – Polish *D. solani* (IFB 0099, IFB 0100, IFB 0158, IFB 0167, IFB 0212), dark blue – Finnish *D. solani* (IFB 0231, IFB 0236, IFB 0254, IFB 0261, IFB 0265), brown – Israeli *D. solani* (IFB 0124, IFB 0125, IFB 0455, IFB 0456, IFB 0457). Bar height represents the mean value from 2 repetitions; the whiskers represent the value of the standard error.

7.1.4 Summary on *Dickeya* spp. phenotypic traits

A summary on phenotypic traits of the tested *Dickeya* spp. strains exhibited at three different temperatures is presented in Table 13.

In general, *D. solani* strains have higher pectinolytic, cellulolytic and proteolytic activities at 18 and 28 °C than *D. dadantii* and *D. dianthicola* strains. At 37 °C *D. dadantii* strains have the highest pectinolytic and proteolytic activity, while *D. solani* strains have the highest cellulolytic activity. Strains of *D. solani* also have a higher total pectate lyses activity in induced conditions than *D. dianthicola*. In general, *D. dianthicola* has the lowest pectinolytic, cellulolytic and proteolytic activities at all tested temperatures.

Among the tested *D. solani* originating from different climatic conditions, Polish strains have the highest pectinolytic, cellulolytic and proteolytic activity at all tested temperatures. They also have a higher total pectate lyses activity in induced conditions than Finnish and Israeli strains. The highest activity of the PCWDE presented by *D. solani* strains isolated in Poland can explain their highest ability to macerate potato tubers tissue.

To answer the question which factor that determines phenotypic traits expression is more important in the production of PCWDE, the coefficients of determination were compared. It was estimated that for enzymatic activities such as pectinases, proteases as well as the ability to chelate iron ions and swarming ability, the species from which strains originate has the higher impact than the temperature. The strains of *D. solani* indicated the highest activities of the mentioned features. In the case of pectinases production, the species impact can explain 69 % of the variation, similarly for swarming 71 %. For proteases species effect explains 52 % of variability and for iron chelating ability 23 %. On the other hand, the influence of the temperature explains only 15 % of the pectinolytic activity variability and only 1 % of proteases and the ability to chelate iron ions. Only for cellulases activity the temperature has a higher impact in comparison to the species from which the strains originate, temperature explains 34 % of variability, while species only 25 %.

As regards the origin of the strains (from which climates the *D. solani* strains were isolated), the temperature has a higher impact on the exhibited traits (pectinolytic, cellulolytic, proteolytic activities as well as motility and ability to chelate iron ions) than the origin itself.

As regards the ability to macerate potato tissue, the origin of the strains plays a more important role than the inoculums level (21 % and 1 % respectively).

The effect of the origin on total pectinolytic activity in induced conditions explains 30 % of the variability.

In conclusion: the species has a higher impact on the strains phenotypic features than the temperature of incubation, however the impact of temperature on the tested traits is significant.

The origin of the strains has a lower impact on phenotypic features than the temperature of their incubation.

Table 13. Summary on phenotypic traits of tested *Dickeya* spp. strains. Pl – pectinolytic activity (qualitative), Cel – cellulolytic activity (qualitative), Prt – proteolytic activity (qualitative), Total Pl – total pectate lyases activity (quantitative), Mac – maceration. Value of the standard error is given in the brackets. nd – not determined.* activity expressed in units: [µmol/min/mg]. Orange – the highest values, blue – the lowest values.

	18 °C					28 °C					37 °C				
	Pl [mm]	Cel [mm]	Prt [mm]	Total Pl	Mac [mm]	Pl [mm]	Cel [mm]	Prt [mm]	Total Pl *	Mac 10^6 [mm]	Pl [mm]	Cel [mm]	Prt [mm]	Total Pl	Mac [mm]
D. solani^{TS}	**8.17 (±0.17)**	2.17 (±0.17)	**4.67 (±0.21)**	nd	nd	**12.00 (±0.26)**	4.67 (±0.21)	**8.50 (±0.22)**	**17.30 (±3.16)**	**19.35 (±0.77)**	6.80 (±0.31)	3.67 (±0.21)	0,00	nd	nd
D. solani Poland (5 strains)	**8.07 (±0.21)**	**2.90 (±0.11)**	**2.92 (±0.19)**	nd	nd	**11.40 (±0.27)**	**8.27 (±0.20)**	**5.07 (±0.17)**	**19.28 (±1.22)**	**20.66 (±0.51)**	**10.73 (±0.22)**	**6.47 (±0.34)**	**0.97 (±0.26)**	nd	nd
D. solani Finland (5 strains)	**7.63 (±0.34)**	**2.73 (±0.12)**	2.41 (±0.25)	nd	nd	**10.83 (±0.40)**	**6.87 (±0.37)**	3.83 (±0.30)	12.32 (±1.12)	16.48 (±0.65)	**8.50 (±0.33)**	5.33 (±0.39)	0.03 (±0.03)	nd	nd
D. solani Israel (5 strains)	5.20 (±0.36)	1.93 (±0.16)	1.28 (±0.20)	nd	nd	9.57 (±0.29)	4.30 (±0.43)	2.80 (±0.39)	5.52 (±1.30)	13.66 (±0.47)	0.65 (±0.53)	4.23 (±0.36)	0.53 (±0.19)	nd	nd
D. solani Mean value for 16 strains	**7.14 (±0.23)**	**2.50 (±0.08)**	**2.34 (±0.15)**	nd	nd	**10.85 (±0.18)**	**6.36 (±0.25)**	**4.19 (±0.22)**	**12.68 (±0.82)**	**17.07 (±0.34)**	**8.60 (±0.26)**	**5.24 (±0.22)**	0.48 (±0.26)	nd	nd
D. dadantii (2 strains)	6.17 (±0.24)	1.17 (±0.11)	1.29 (±0.11)	nd	nd	**10.92 (±0.23)**	3.92 (±0.40)	3.17 (±0.11)	**11.66 (±2.60)**	nd	**10.08 (±0.23)**	4.42 (±0.15)	**3.00 (±0.25)**	nd	nd
D. dianthicola (3 strains)	1.94 (±0.37)	1.44 (±0.17)	0.00	nd	nd	4.56 (±0.49)	2.11 (±0.18)	0.22 (±0.15)	0.01 (±0.003)	nd	0.17 (±0.11)	2.22 (±0.15)	0.00	nd	nd

7.1.5 Molecular profiling of *D. solani* strains from different climatic conditions

Two molecular profiling techniques: rep-PCR and RFLP-PFGE were used to indicate differences in genomic material of Polish, Finnish and Israeli strains that could be responsible for different phenotypes of the tested strains. Fingerprinting with rep-PCR method is a common method applied to present differences between closely related species and strains of bacteria on the basis of dispersed repetitive DNA sequences distribution (such as Repetitive Extragenic Palindromes – REP, Enterobacterial Repetitive Intergenic Consensus sequences – ERIC and BOX). RFLP-PFGE is a gold strandard method for discriminating molecular profiles of closely related microorganisms. The profiles were visually evaluated. In Figures 33-36, the molecular profiles obtained on the basis of rep-PCR and RFLP-PFGE are presented. The observed genomic profiles of all tested *D. solani* strains are the same regardless of the profiling method used. Profiles of *D. solani* from Poland, Finland and Israel have the same molecular pattern as *D. solani*^{TS}. At the same time they do differ from the profiles of *D. dadantii* (strains IFB 0010 and IFB0016) and *D. dianthicola* (strains IFB 0103 and IFB 0157). In Figure 36, the profile of *D. solani*^{TS}is not presented, but profile identities of*D. solani*^{TS}, *D. solani* IFB 0099 and IFB 0158, as well as 34 other Finnish strains were shown earlier (Degefu et al. 2013).

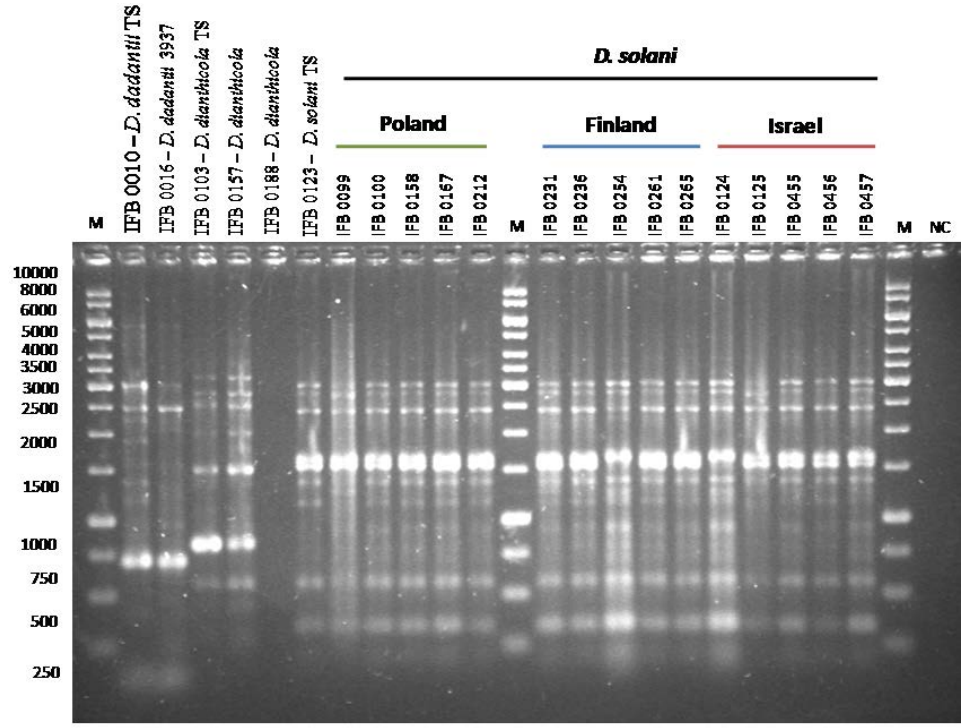

Figure 33. Molecular profiles of *Dickeya* spp. obtained by REP-PCR.M – marker 1 kb from Fermentas, NC – negative control – water.

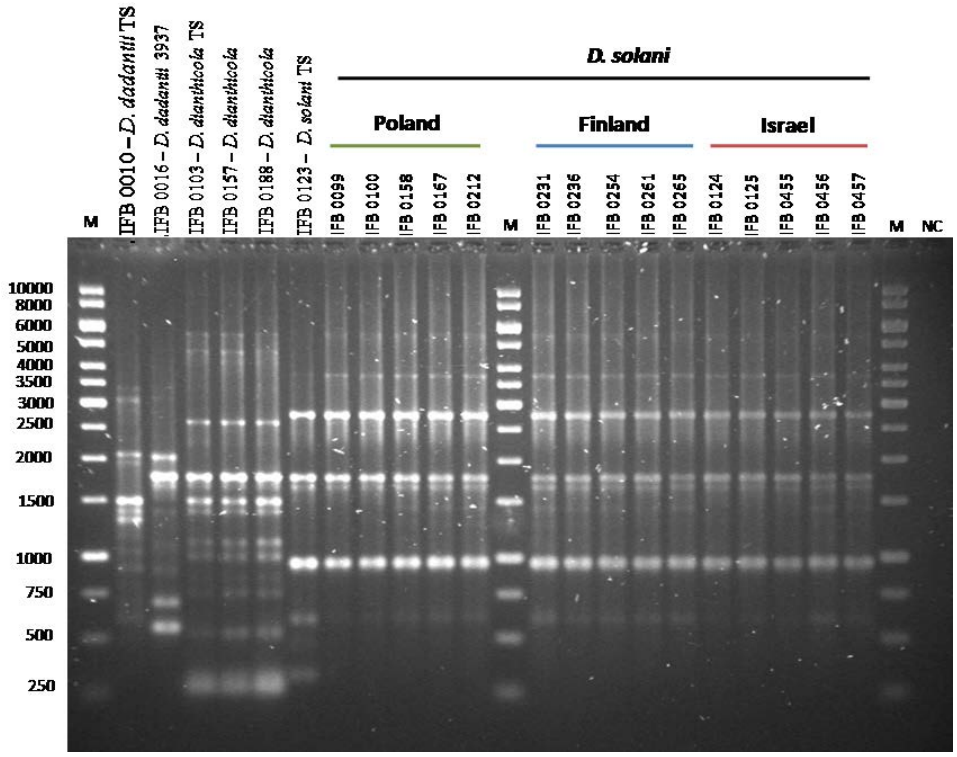

Figure 34. Molecular profiles of *Dickeya* spp. obtained by ERIC-PCR. M – marker 1 kb from Fermentas, NC – negative control.

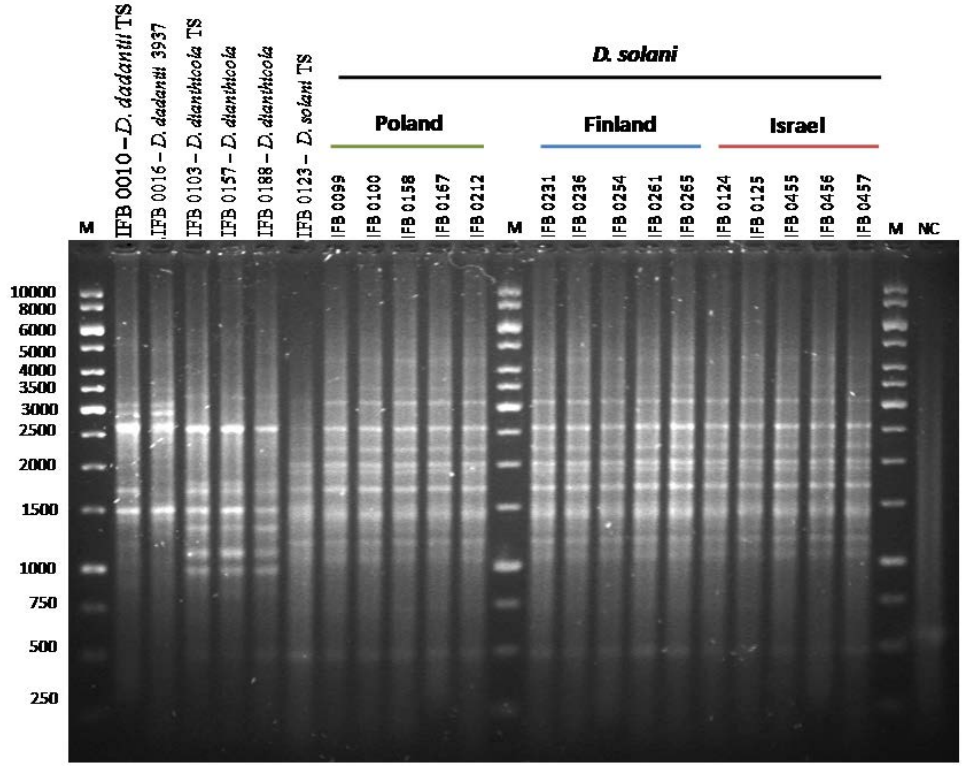

Figure 35. Molecular profiles of *Dickeya* spp. obtained by BOX-PCRM – marker 1 kb from Fermentas, NC – negative control.

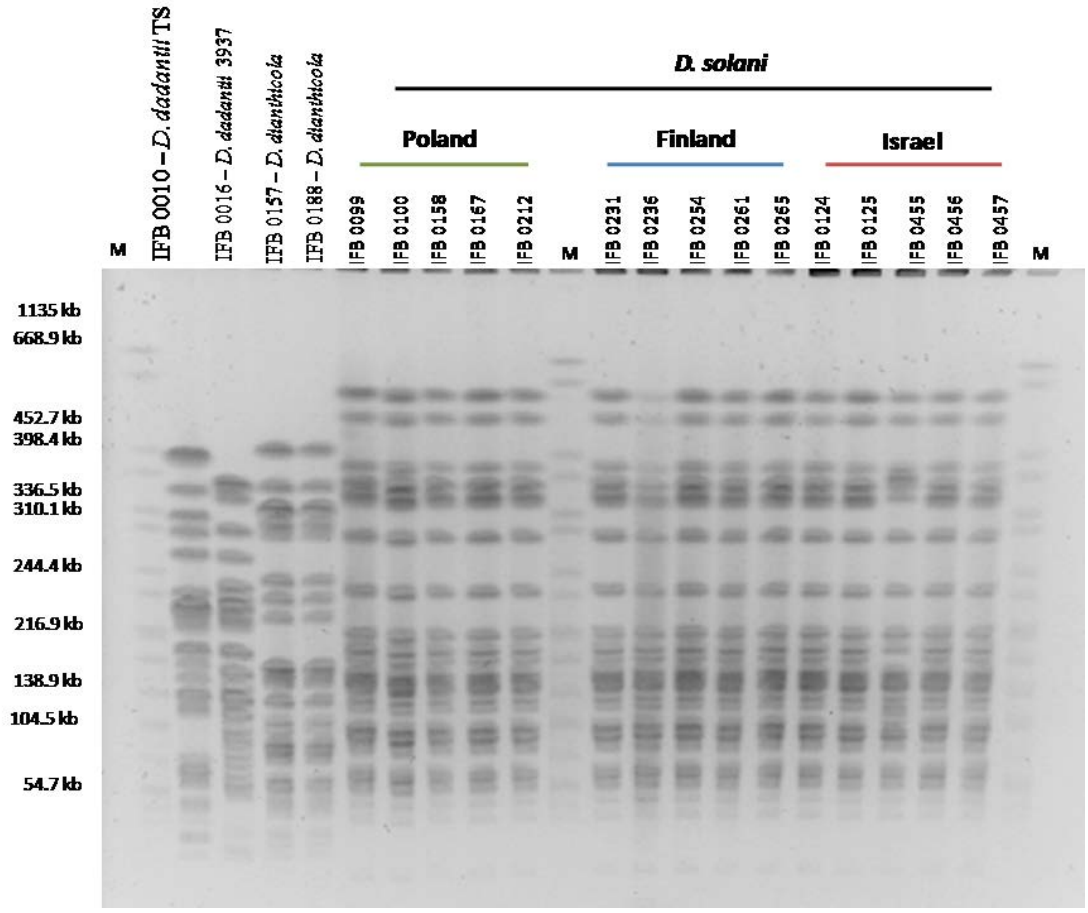

Figure 36. Molecular profiles of *Dickeya* spp. by PFGE. M – marker – *Salmonella enterica* serovar Braenderup ATCC H9812.

The purpose of genetic fingerprinting is to evaluate the relative degrees of similarity and evaluate if strains are clonally related. All of the tested *D. solani* strains have the same REP, ERIC and BOX profiles as well as PFGE profile. This can indicate that *D. solani* strains are related to each other and their genomic structure is highly similar. The conclusion is that *D. solani* strains even though originating from different geographic and climatic regions are very closely related and phenotypedifferences between the tested *D. solani* strains cannot be explained by their genomes diversity.

7.2 Influence of potato tissue extract on the total pectynolytic activity of *D. solani* strains and expression of genes important for the diseases symptoms development

The aim of this part of study was to check the influence of potato tissue extract on pectinolytic activity of *D. solani* strains and to find the plant tissue compound/s that induces pectinolytic activity, important for disease symptoms development.

Firstly, the impact of potato tuber extract on total the pectate lyases activity was tested on *D. solani* IFB 0099 and the *D. solani*^{TS} (IFB 0123), (Figure 37. Induction of total pectate lyases activity of *D. solani* strains by PGA). The pectinolytic activity was measured for each strain growing under 4 different conditions (minimal medium M63 Y – non-inducing conditions; M63 Y supplemented with 2 % PGA – inducing conditions; M63 Y supplemented with 1% potato tuber extract (PE) and M63 Y supplemented by both – 2 % PGA and 1 % PE). The total pectinolytic activity was measured spectrophotometrically (Methods 6.6.15).

The results show that the total pectate lyses activity in both tested *D. solani* strains is highly induced by PGA (on average 11 fold, Table 14). The induction of total pectate lyases activity of *D. solani* IFB 0099 and IFB 0123 strains by PE was 3 to 4 times lower than its induction by PGA. The pectate lyases activity was induced by PE only about 3 fold. For *D. solani* IFB 0123 the induction ratio for PE was about 2 fold higher than for *D. solani* IFB 0099, but still 3 times lower that those obtained with PGA.

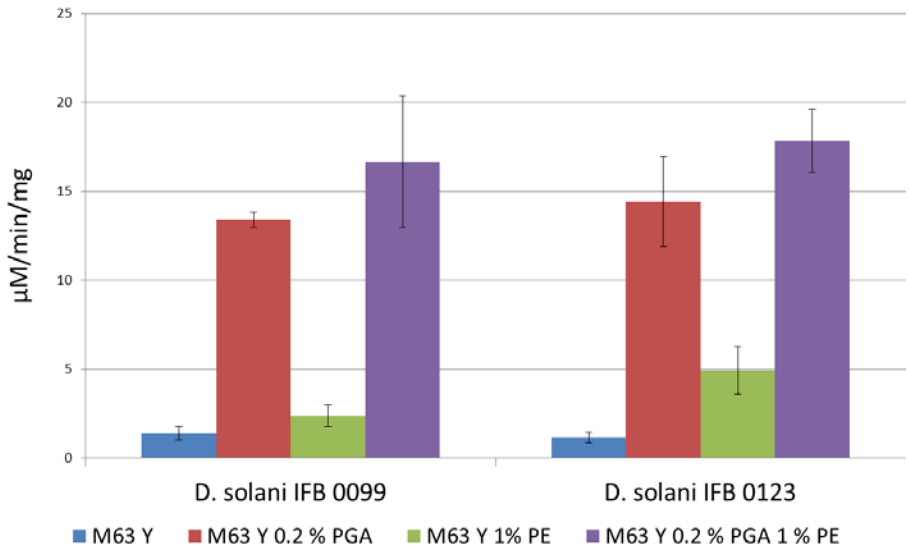

Figure 37. Induction of total pectate lyases activity of *D. solani* strains by PGA, PE and PGA with PE. Bar height represents the mean value from 3 repetitions; the whiskers represent the value of the standard deviation.

In conditions where the medium was supplemented by both: PGA and potato tuber extract, the pectate lyases activity was the highest for both tested strains, but differences were not significant in comparison to the level of PGA induction.The induction ratios for all tested conditions are presented in Table 14.

Table 14.Comparison of the induction ratios of pectinolytic activity for *D. solani* strains incubated in different conditions.

Induction ratio	M63 Y 0.2 % PGA	M63 Y 1 % PE	M63 Y 0.2 % PGA 1 % PE
D. solani IFB 0099	9.70	1.72	12.08
D. solani IFB 0123	12.59	4.31	15.54

Since the results of the total pectate lyses activity induction by crude plant extract were not satisfying, a different approach to studying the influence of potato extract was applied. It was decided to perform site-specific mutagenesis in selected genes to check if the expression of these genes is induced with potato extract. The genes for mutagenesis were chosen on the basis of literature that implicated an induction impact of plant tissue on bacterial gene expression. Finally, for the mutagenesis, the following genes from three groups were chosen.

The first group was represented by two genes encoding pectinolytic enzymes (main virulence factors), one of them being a representative of the main and the other of the secondary pectate lyases, *pelD* and *pelL*, respectively (Berteau et al.1984, Lojkowska et al. 1995). The second group was represented by one of the T6SS structural genes – *tssK* (Mattinen et al., 2008; synonymous name in *D. dadanti* 3937 –*impJ*). And the third gene *lfaA* is an element under control of LacI family regulator (Van Gijsegem et al. 2008).

For studying the expression of the selected genes, the site-specific mutagenesis was applied, by introducing the cassette of the reporter gene – β-glucuronidase (GUS, *uidA*) and the gene encoding resistance against chloramphenicol (cm^R) or kanamycin (kan^R) into the gene of interest. When the reporter gene is expressed in the tested conditions (e.g. induction of expression by PGA or PE) the enzyme GUS is produced and its activity is measured spectrophotometically (Methods 6.6.16.).

Firstly, the PCR product of the gene of interest was cloned into the p-GEM plasmid. After the restriction digestion of the plasmid, the cassette with GUS and the antibiotic resistance genes were inserted into the gene of interest and the plasmid was introduced into the cells of *E. coli* NM 522 by transformation and multiplied. The presence and orientation of the obtained product was checked after plasmid isolation and its restriction enzyme analysis. Later, the constructed plasmid was introduced by electroporation to *D. dadantii*3937. Then on the basis of homological recombination, the cassette was built-in to *D. dadantii*3937 genome. After obtaining the mutant in *D. dadantii*3937, the ØEC2 phage stock was prepared. *D. solani* mutants were obtained by the means of transduction with the use of ØEC2 DNA containing the gene of interest with the GUS cassette. The same procedure to obtain mutants in *pelD, pelL, tssK* and *lfaA* genes was performed. All the obtained transductants were confirmed by PCR regarding the presence of the cassete and on selective media (M63 Y PGA, M63 Y CMC, milk agar) for evaluating their phenotypical properties. Below, the results of the study of the potato tuber extract influence on selected genes expression are presented.

The above described part of the study was conducted in a laboratory of my co-supervisor Dr. Nicole Hugouvieux-Cotte-Pattat (MAP, INSA Lyon), because of her expertise in the work with *Dickeya dadantii* 3937 and the collection of *D. dadantii* 3937 mutants available in her laboratory.

7.2.1 Influence of potato tuber extract on the expression of gene encoding *pelD* gene, the main pectate lyase

Pectate lyases are the most important pathogenicity factors for *Dickeya* species. The knowledge how pectinolysis is regulated and induced in *D. solani* is poor. We only possess knowledge of how 3 main regulators of pectinolysis (KdgR, PecS and PecT) act in *D. solani* in cells induced by PGA, there is no information about the influence of potato plant extract on the expression of the pectinolytic enzymes genes. The main pectate lyases in *D. dadantii* are known to be uninfluenced by crude potato tuber extract, but the goal of my work was to check if it is also true for *D. solani*. The induction of the expression of *D. solani* genes coding for the main pectate lyases by crude potato tuber extract can explain why *D. solani* has a higher pectinolytic activity than *D. dianthicola.* For manipulation, *pelD*, one of the main pectate lyases genes, was chosen. The existence of the *pelD* gene in *D. solani* genome was confirmed by local Blast implemented in BioEdit software. The sequence of *pelD* gene of *D. dadantii* 3937 was aligned to the genomic sequence of the *D. solani* IFB 0123. The fished out sequence was the same length as in *D. dadantii* 3937 and showed 89 % of identity.

GUS activity was evaluated for each mutant in three different media: M63 Y (non- induced conditions); M63 Y supplemented with 0.2 % PGA (induced conditions) and M63 Y supplemented with 1 % PE (induced conditions).

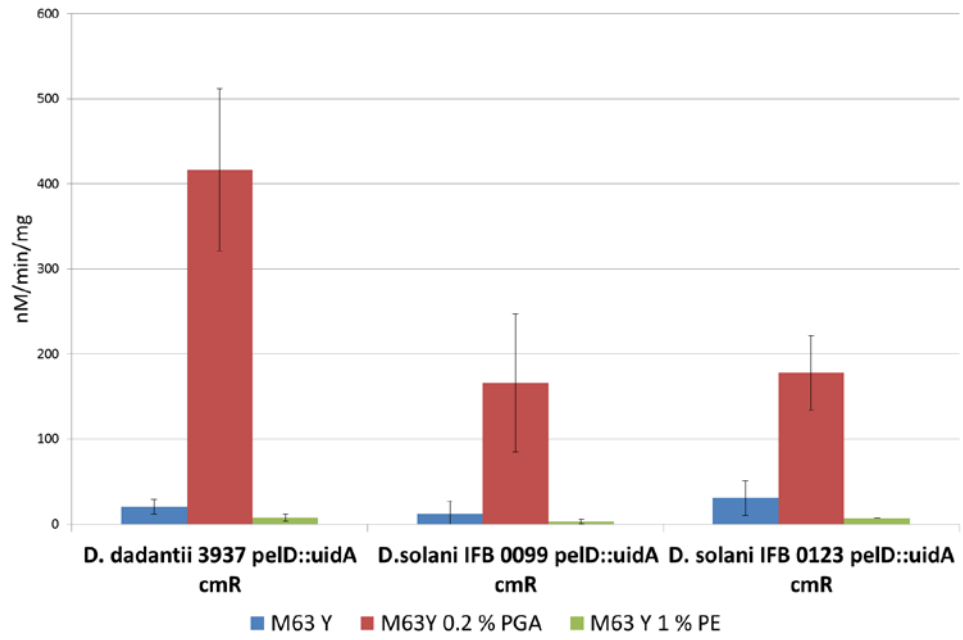

Figure 38. The influence of the PGA and PE on *pelD* gene expression in *D. dadantii* and *D. solani*cells.Bar height represents the mean value from 3 repetitions; the whiskers represent the value of the standard deviation.

Table 15. The induction ratio of *pelD* gene expression in *D. dadantii* and *D. solani* mutants growing in a medium supplemented with PGA or PE.

Induction ratio:	M63Y 0.2%PGA	M63Y 1%PE
D. dadantii 3937 *pelD*::uidA Cm^R	20.33	0.37
D.solani 99 pelD::uidA Cm^R	13.74	0.25
D. solani 123 pelD::uidA Cm^R	5.75	0.24

The results indicated the induction of *pelD* by PGA. The gene expression of *pelD* for *D. dadantii* 3937 was induced 20 times, for *D. solani* IFB 0099 almost 14 times and for *D. solani* IFB 0123 only almost 6 times (Table 15).

There is no inducing impact of the potato tuber extract on *pelD* gene expression both for *D. dadantii* and for *D. solani* (Figure 38, Table 15).

7.2.2 Influence of potato tuber extract on secondary pectate lyase *pelL* gene expression in vitro

The secondary pectate lyases play a role in the tuber tissue maceration by *D. dadantii* 3937 and according to our knowledge its activity is especially important in the first phase of infection. It can be induced by plant extract (Lojkowska et al., 1995). For mutagenesis gene *pelL* was chosen. The presence of the gene in *D. solani* genome was confirmed by local Blast implemented in BioEdit software. The sequence of *pelL* gene of *D. dadantii* 3937 was aligned to the genomic sequence of the *D. solani* Type Strain IFB 0123 (IPO 2222). The fished out sequence was the same length as in *D. dadantii* 3937 and showed 92 % of identity. A few attempts for obtaining the mutants in *D. solani* strain IFB 0099 were performed, but none of them was succesful. That is why the results are presented only for strains *D. dadantii* 3937 and *D. solani* IFB 0123 (Figure 39).

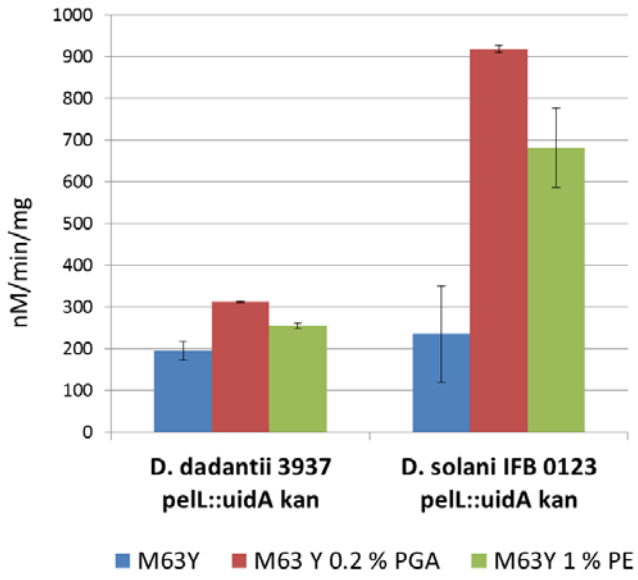

Figure 39. The influence of PGA and PE on *pelL* gene expression in *D. dadantii* 3937 and *D. solani* IFB 0123. Bar height represents the mean value from 3 repetitions; the whiskers represent the value of the standard deviation

There is a higher impact of potato tuber extract on *pelL* gene expression in *D. solani* than in *D. dadantii* (induction ratio 2.9 versus 1.3 respectively, Table 17). But the induction ratio in the medium with PGA was higher than in those with PE.

Table 16. Induction ratio for *pelL* mutants in *D. dadantii* and *D. solani* growing in medium supplemented with PGA or PE.

Induction ratio	M63 Y 0.2 % PGA	M63 Y 1% PE
D. dadantii 3937 *pelL*::uidA kan^R	1.6	1.3
*D.solani*IFB0123 *pelL*::uidAkan^R	3.9	2.9

7.2.3 Influence of potato tuber extract on type VI secretion system component *tssK* gene expression *in vitro*

Recently the genomic study performed on the French strain *D. solani* 3337 (RNS08.23.3.1A) has shown that T6SS can have a special role in virulence of *D. solani* (Pedron et al., 2014). But there is no information how this system is regulated in *D. solani* cells and if the plant extract induces the expression of the genes forming T6SS cluster. The T6SS was also studied in *P. atrosepticum* (Mattinen et al., 2008). It was found on the basis of the microarray analysis that the structural genes of T6SS are induced by plant extract. For the mutagenesis, gene *tssK* was chosen. The presence of the gene in *D. solani* was confirmed by Local Blast implemented in BioEdit software. The sequence of *tssK* gene of *D. dadantii* 3937 was aligned to the genomic sequence of the *D. solani* Type Strain IFB 0123 (IPO 2222).The fished out sequence was the same length as in *D. dadantii* 3937 and showed 97 % of identity.

The mutant strain of *D. dadantii* in *tssK* gene was grown in 3 different conditions: M63 Y (non-induced conditions); M63 Y supplemented with 0.2 % PGA (induced conditions) and M63 Y supplemented with 1% PE (induced conditions) (not shown). There was no induction of tssK expression in either PGA or potato tuber extract in *D. dadantii* 3937. Nevertheless, mutants of *D. solani* in *tssK* gene were tested under non-inducing and inducing conditions (M63 Y and M63 Y supplemented with 1% PE. The result of the potato tuber extract impact on *tssK* gene expression is presented in Figure 40.

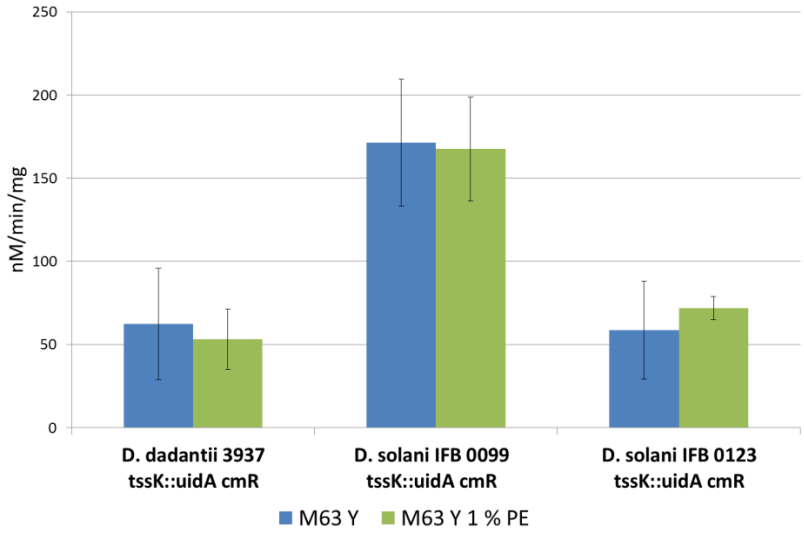

Figure 40. The influence of PE on *tssK* gene expression in *D. dadantii* and *D. solani* mutants. Bar height represents the mean value from 3 repetitions; the whiskers represent the value of the standard deviation

The obtained results indicated that there was no inducing impact of potato tuber extract on *tssK* gene expression either in *D. dadantii* 3937 or in both *D. solani* strains.

7.2.4 Influence of potato tuber extract on LacI family - *lfaA* gene expression in vitro

LfaA gene has been chosen on the basis of information showing the influence of plant extract on this gene expressionin the cell of *D. dadantii* 3937 (van Gijsegem et al. 2008). Van Gijsegem and colleagues (2008) examined 7 of the 18 LacI regulators in *D. dadantii* 3937 for which no specific function was known and found that genes under control of four of these seven regulators are expressed during plant infection. That study showed the influence of crude potato tuber extract on the expression of the genes under control of three of the studied regulators (*lfa, lfc, lfe*). So, the impact of the plant extract on this gene expression was studied in *D. solani*. One of the representative genes was chosen for study in *D. solani* – *lfaA*. The existence of the gene was confirmed by local Blast implemented in BioEdit software. The sequence of *lfaA* gene from *D. dadantii* 3937 was aligned to the genomic sequence of the *D. solani*^{TS} and showed 93 % of identity.

lfaA mutants were grown in three different media conditions: M63 Y (non- induced conditions), M63 Y supplemented with 0.2 % PGA (induced conditions) and M63 Y supplemented with 1 % PE (induced conditions). There is a high inducing impact of PE on

lfaA gene expression in *D. dadantii* 3937 and *D. solani* strains IFB 0099 and IFB 0123. Induction of the *lfaA* expression in *D. solani* IFB 0123 is the highest. Interestingly, PGA does not induce the *lfaA* gene in both *D. dadanti* and *D. solani* background (Figure 41). The induction ratio in the tested conditions is presented in Table 17.

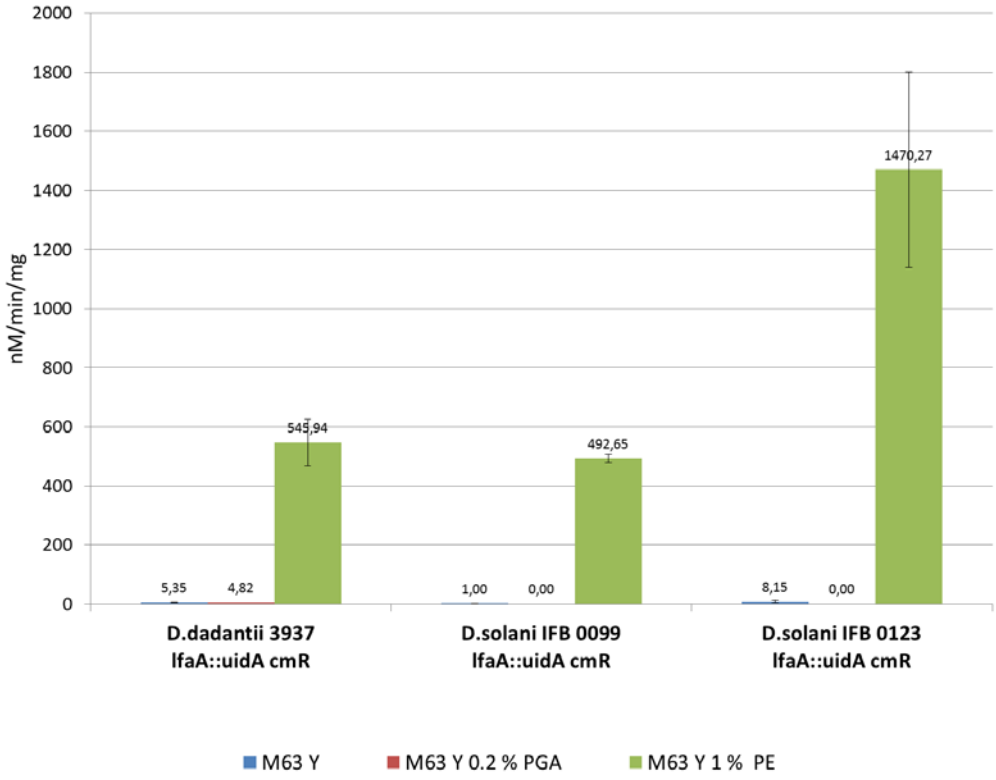

Figure 41. The influence of the PGA and PE on *lfaA* gene expression in *D. dadantii* and *D. solani* mutants. Bar height represents the mean value from 3 repetitions; the whiskers represent the value of the standard deviation

Table 17. Induction ratio for mutants in *D. dadantii* and *D. solani* studied in a medium supplemented with tuber PE.

Mutants in *lfaA* gene	M63 Y 0.2 % PGA	M63 Y1%PE
D. dadantii 3937 *lfaA*::*uidA* cm^R	0.90	102.00
*D.*solani IFB 0099 *lfaA*::*uidA* cm^R	0	492.65
D.solani IFB 0123 *lfaA*::*uidA* cm^R	0	180.31

In conclusion: the expression of genes selected for the presented study in two *D. solani* strains IFB 0099 and IFB 0123 is similar to the expression of the same genes in *D. dadantii* 3937 (besides *pelL*, where the induction by plant extract in *D. solani* IFB 0123 is higher than

in *D. dadantii* 3937). The *lfaA* gene (from LacI family) is induced by plant extract exclusively. The supplementation of the media with PGA has no effect on *lfaA* gene expression. The potato tuber extract has no inducing influence on neither *pelD* nor *tssK* genes in *D. solani* strains and in *D. dadantii* 3937.

Even though the main goal of the study was to find the plant derivative compounds that induce the pectinolytic activity, the goal has not been achieved yet. The search of the inducing compound will be continued. The study presented here, especially concerning induction of the *pelL* and *lfaA* gene expression by plant extract, constitutes a start for further detailed research.

7.3 Comparative genomics of selected *D. solani* strains

This part of the study was dedicated to the analysis of the genomic diversity within *D. solani* species. The 10 *D. solani* genomic sequences were used to establish the pangenome shape. The study included 4 *D. solani* strains analyzed earlier in our Laboratory that exhibited different virulence levels (2 strains isolated in Poland from symptomatic potato plant that show a high level of virulence and 2 strains isolated in Germany from potato plant rhizosphere that exhibit a low level of virulence). In addition, six other genomic sequences already available in GenBank database were used (available in November 2014 when the analysis was performed). The syntheny (genome structure) of the strains as well as their phylogeny was studied. LS-BSR method was applied to establish if the sequences of genes encoding pectinolytic and cellulolytic enzymes (altogether 9 enzymes) and genes coding for 19 regulation-related proteins are present and the homology of their regulators was analyzed.

This part of the work was done in collaboration with the group of Prof. Marco Bazzicalupo from the University of Florence.

7.3.1 General features of the analyzed genomes

General statistical information about *D. solani* genomes has been gathered in Table 19. The length of the analyzed genomes is between the range from 4 812 070 to 5 095 081 bp. The average GC content is between 55.92 and 56.40 %. The ORF numbers vary from 4 161 to 4 519.

7.3.2 Structural genomics

The six strains (IFB0099, IFB0223, IFB0158, IFB0221, RNS08.23.3.1A and D s0432-1) have been aligned to the *D. dadantii* 3937 genomes sequence using the CONTIGuator web server (version 2.7.3;e-value 1e-5, hit length threshold 250, contig coverage threshold 5%) to obtain the scaffolds of the sequences for comparison purposes. The remaining four sequences were already at the scaffold or chromosome stage of assembly. The scaffolded sequences were used to study the syntheny of the selected genomes. The syntheny of 10 tested genomes is presented in Figure 42. The strains were compared sequentially one to another. For example, the sequence of GBBC 2040 was aligned to the sequence of strain D s0432 and this sequence was aligned to the sequence of IFB 0158 *et cetera*. The strains are structurally similar to each other, but small rearrangements between them such as inversions (in dark red) and translocations (in blue) are observed within strains. Strains RNS 08.23.3.1A (IPO 3337, isolated in France) and D s0432-1(isolated in Finland) appear to be structurally more distant from the other strains, showing a slightly larger number of regions that are inverted or translocated in the other strains.

Table 18. General features of *D. solani* genomes.* The genomes sequences that are already published have been analyzed again for the purpose of this study with the same tools as the unpublished, so the values can differ from the published.

***D. solani* strains**	**Origin**	**Analyzed by**	**Length (bp)**	**GC%***	**ORF***
IFB0099	Poland, potato	Golanowska et al., 2015	5 095 081	56.35	4 365
IFB 0158	Poland, potato	This work	4 889 604	56.25	4 161
IFB 0221	Germany, potato rhizosphere	This work	4 903 613	56.22	4 169
IFB0223	Germany, potato rhizosphere	This work	4 865 597	55.92	4 325
IPO2222 TS	The Netherlands, potato	Pritchard et al., 2013	4 857 348	56.20	4 471
GBBC2040	Belgium, potato	Pritchard et al., 2013	4 812 070	56.40	4 471
MK10	Israel, potato	Pritchard et al., 2013	4 930 219	56.20	4 519
MK16	UK- Scotland, river water	Pritchard et al., 2013	4 867 774	56.19	4 401
D s0432-1	Finland, potato	Garland et al., 2013	4 900 000	56.20	4 173
RNS 08.23.3.1A (IPO 3337)	France, potato	Khayi et al., 2014	4 923 734	56.11	4 337

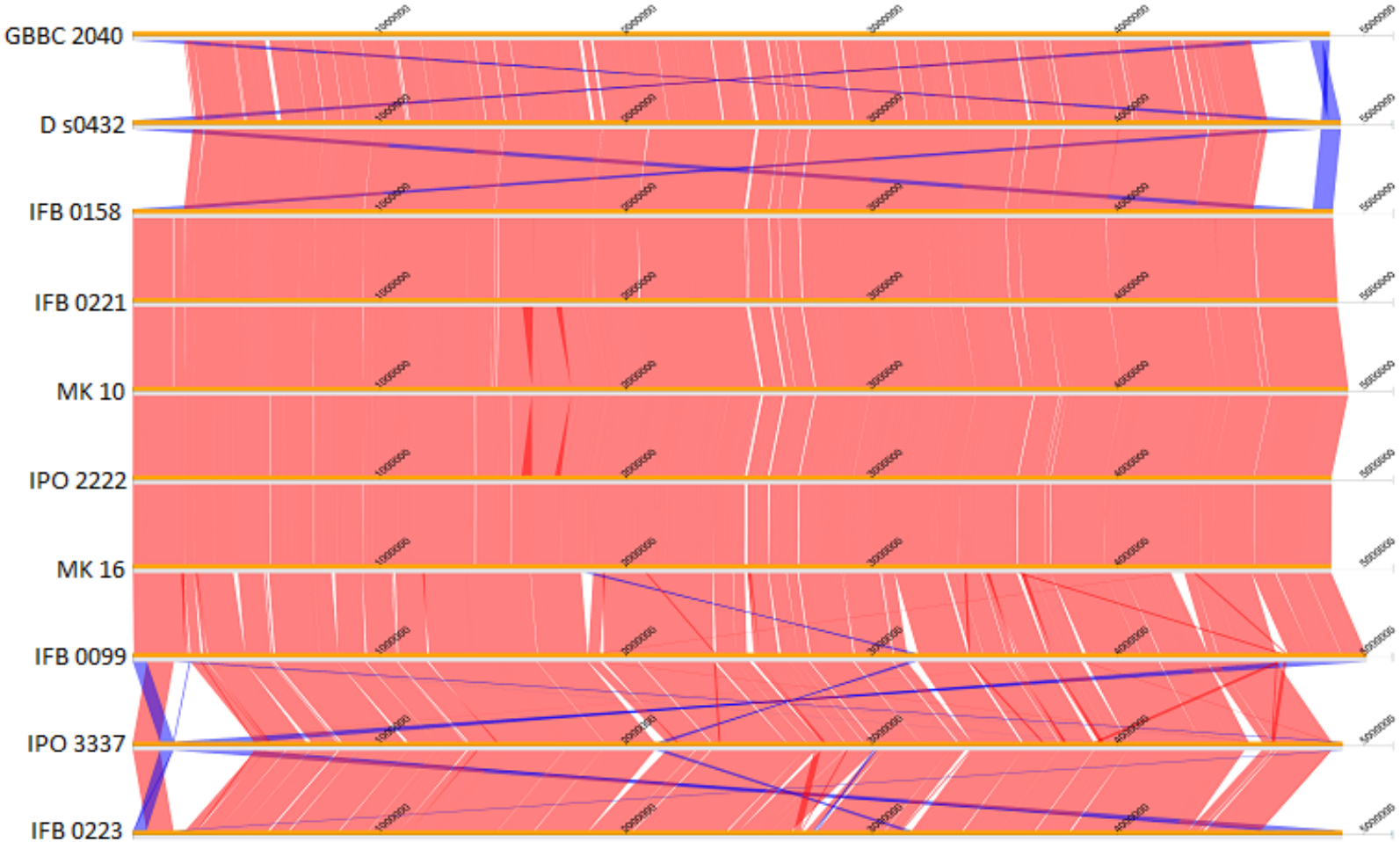

Figure 42. The syntheny of the analyzed *D. solani* genomic sequences. The genomic alignment has been visualized using the genome diagram package inside BioPython (version 1.62b).

7.3.3 Orthology assessment

The orthology relationships inside the *D. solani* pangenome have been computed using the Blast-BBH algorithm implemented in the DuctApe suite (version 0.16.2). The 41 947 total proteins belonging to the 10 strains have been grouped into 5 045 Orthologous Groups (OGs), 3 809 (75.5 %) belonging to the core genome (present in all 10 genomes), 413 to the accessory genome (present in more than one and less than 10 genomes in this case, it comprises 8.2 % of the pangenome genes) and 823 to the unique genome (present only in single genomes, it comprises 16.3 % of pangenome). The pangenome shape of *D. solani* is presented in Figure 43.

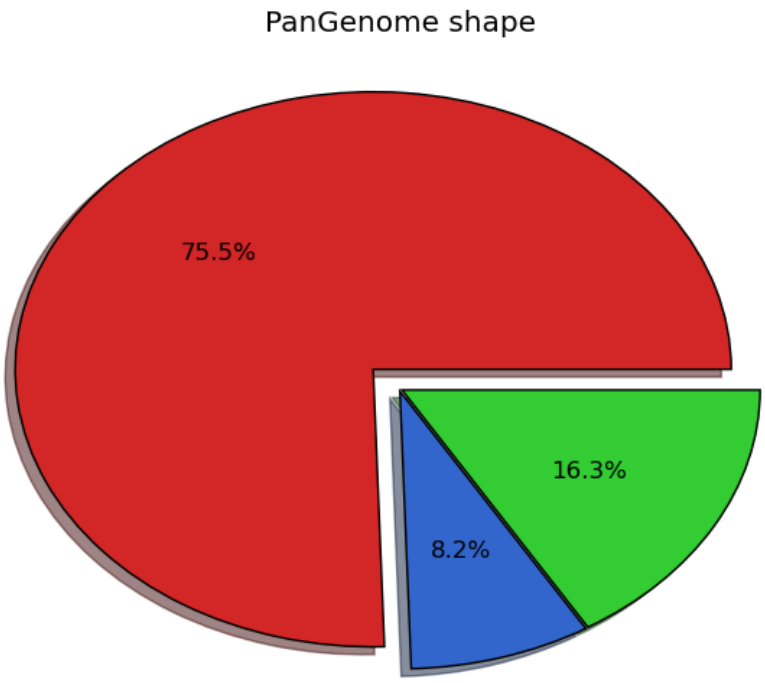

Figure 43. *D. solani* pangenome shape. Red – core genome, green – unique genome, blue – accessory genome.

In the *D. solani* core genome the presence of housekeeping genes such as *dnaX, recA, dnaN, fusA, gapA, purA, rplB, rpoS* and *gyrA* has been confirmed. Also the main pathogenicity related proteins and regulators of pectinolysis have been confirmed to be present in *D. solani* core genome. Table 19 shows arbitrarily chosen genes present in *D. solani* core genome and their function. Genes involved in disease development (listed in Table 2) such as *bcsABCD, wza-wzb-wzc, motABcheAWDRBYZ, sapABCDF, hrpA-hrpE, hrpN, budAB, pelA-pelE-pelD, pelB-pelC, pelL, celZ, prtG-inhprtDEFBCA* have also been annotated (examples presented in Table 19).

Table 19. Examples of genes involved in disease development present in *D. solani* core genome.

Gene name	Function
arnC	Undecaprenyl-phosphate 4-deoxy-4-formamido-L-arabinose transferase
arnA	Polymyxin resistance protein PmrI;Bifunctional polymyxin resistance protein ArnA, putative
arnD	putative protein yfbH;putative 4-deoxy-4-formamido-L-arabinose-phosphoundecaprenol deformylase ArnD
arnE	putative 4-amino-4-deoxy-L-arabinose-phosphoundecaprenol flippase subunit ArnE;Undecaprenyl phosphate-aminoarabinose flippase subunit ArnE
arnF	putative 4-amino-4-deoxy-L-arabinose-phosphoundecaprenol flippase subunit ArnF;Undecaprenyl phosphate-aminoarabinose flippase subunit ArnF
arnT	Undecaprenyl phosphate-alpha-4-amino-4-deoxy-L-arabinose arabinosyl transferase
arnB_2;arnB_1	UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotransferase;TDP-4-keto-6-deoxy-D-glucose transaminase
arnB_2;arnB_1	UDP-4-amino-4-deoxy-L-arabinose-oxoglutarate aminotransferase;UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotransferase;UDP-4-amino-4-deoxy-L-arabinose alpha-ketoglutarate aminotransferase
sapC	Peptide transport system permease protein SapC
hcaR_1;hcaR_3	Hca operon transcriptional activator;HTH-type transcriptional regulator BudR (Bud operon transcriptional regulator)
pemA	Pectinesterase A precursor;Pectinesterase A
pelA_3;pelA_2;pelA_4	Pectate lyase A;Pectate lyase A precursor
pelE_4;pelE_2;pelE_1	Pectate lyase E precursor;Pectate lyase D
pelE_5;pelE_2;pelE_1	Pectate lyase E precursor;Pectate lyase E
prtS	Grimelysin;Protease PrtS precursor
prtB	Serralysin;Serralysin B precursor
prtC_2;prtC;prtC_1	Serralysin;Serralysin C precursor
prtA;prtC_2;prtC_1	Serralysin;Serralysin A precursor;Serralysin C precursor
ohrB	General stress protein 17o;Organic hydroperoxide resistance protein ohrB, osmC family
ohrR	Organic hydroperoxide resistance transcriptional regulator
hrpN	Harpin-Ea;Hairpin protein N
hrpB_1;hrpB_2	ATP-dependent RNA helicase HrpB;ATP-dependent RNA helicase HrpA
hcpA_4;hcpA_5;hcpA_7;hcpA_3;hcpA_2	Hcp;Secreted protein hcp
motA	Chemotaxis protein motA;Chemotaxis protein MotA
cheY_1;cheY_2	Chemotaxis protein CheY;Response regulator of RpoS
celZ	Endoglucanase Z precursor;beta-1,4-endoglucanase
celY	Endoglucanase (Endo-1,4-beta-glucanase Y);Minor endoglucanase Y precursor
pksL_4;pksL_3;pksL_2	Polyketide synthase PksL;FkbH like protein;FkbH
pksE_1;pksE_2	Polyketide biosynthesis protein PksE;Putative Glutamate-1-semialdehyde 2,1-aminomutase
pksH	putative polyketide biosynthesis enoyl-CoA hydratase PksH;polyketide biosynthesis enoyl-CoA hydratase;Putative polyketide biosynthesis

	enoyl-CoA hydratase, pksH-like protein
pksF	Putative polyketide beta-ketoacyl synthase;Polyketide biosynthesis malonyl-ACP decarboxylase PksF
pksJ_5;pksN;pksN_1	Polyketide synthase PksN;6-deoxyerythronolide-B synthase;hypothetical protein;Polyketide synthase PksJ
pksJ_2;pksM_2	Polyketide synthase PksM;KR domain protein;hypothetical protein;Polyketide synthase PksJ
pksC;baeC_2	Polyketide biosynthesis malonyl CoA-acyl carrier protein transacylase PksC;BryP;Polyketide biosynthesis malonyl CoA-acyl carrier protein transacylase BaeC
pksE_1;pksE_2	PfaD family protein;Polyketide biosynthesis protein PksE;permease
pksL_1;pksL_2	Polyketide synthase PksL;Mycocerosate synthase, 6-deoxyerythronolide-B synthase;hypothetical protein
wzc_2;wzc_1	Capsular polysaccharide synthesis enzyme CpsD exopolysaccharide synthesis;Tyrosine-protein kinase wzc
wzc_2;wzc_1	Exopolysaccharide tyrosine kinase;Tyrosine-protein kinase wzc
wzb_1;wzb	Low molecular weight protein-tyrosine-phosphatase wzb;protein-tyrosine phosphatase;Protein-tyrosine phosphatase Wzb
nifA	Nif-specific regulatory protein
nifF	Flavodoxin-2;Flavodoxin
nifS	Cysteine desulfurase NifS;Cysteine desulfurase
nifU_1;nifU_2	NifU-like protein;Nitrogen fixation protein nifU
nifH	Nitrogenase iron protein
nifJ_1;nifJ_2	Pyruvate-flavodoxin oxidoreductase
nifB	Nitrogenase cofactor biosynthesis protein NifB;FeMo cofactor biosynthesis protein NifB
nifL_2;nifL	Nitrogen fixation regulatory protein
nifD_2;nifD_1	Nitrogenase molybdenum-iron protein alpha chain;Nitrogenase protein alpha chain
nifK_1;nifK_2	Dinitrogenase beta subunit;Nitrogenase molybdenum-iron protein beta chain
nifK_1;nifK_2	Nitrogenase molybdenum-iron cofactor biosynthesis protein NifN;Nitrogenase molybdenum-iron protein beta chain
nifD_2;nifD_1	Nitrogenase MoFe cofactor biosynthesis protein;Nitrogenase molybdenum-iron protein alpha chain
nifJ_2;nifJ_1	Pyruvate-flavodoxin oxidoreductase
nifU_2;nifU_1	NifU-like protein;FeS cluster assembly scaffold IscU
sufS	Cysteine desulfurase
sufD	FeS cluster assembly protein SufD;Required for stability of iron-sulfur component of FhuF
sufE	Cysteine desulfuration protein sufE;cysteine desufuration protein SufE;Cysteine desulfuration protein SufE
ftnA	Cytoplasmic ferritin;Ferritin-1;ferritin
sufC	putative ATP-dependent transporter SufC;FeS assembly ATPase SufC
sufB	Component of SufBCD complex;FeS cluster assembly protein SufB
iscA_2;iscA_1;sufA	iron-sulfur cluster assembly scaffold protein;Iron-sulfur cluster assembly scaffold protein;Iron-sulfur cluster assembly protein
iscR	HTH-type transcriptional regulator IscR;HTH-type transcriptional regulator iscR
iscS	Cysteine desulfurase
iscA_2;iscA_1;iscA	iron-sulfur cluster assembly protein;Iron-sulfur cluster assembly protein

A sequence of genes encoding proteins involved in nitrogen fixation, iron homeostasis and a set of polyketide synthases are also found in the core genome of *D. solani*.

When it comes to the accessory genome of *D. solani*, there are 73 combinations of it comprising 413 ORFs. The composition of the accessory genome and its combinations and ORFs included in each of them are summed up in Table 20.

Table 20. Accessory genome of *D. solani.* In description shortcuts of strains used as follows: 158 – IFB 0158; 2040 – strain GBBC2040; 221 – strain IFB 0221; 2222 – strain IPO 2222; 223 – strain IFB 0223; 99 – strain IFB 0099;MK 10 – strain MK 10; MK 16 – strain MK 16; IPO 3337 – strain RNS 08.23.3.1A; s0432 – strain D s0432-1.

Accessory genomes sets	**Number of accsserory ORFs**	**Number of hypothetical proteins**	**Number of annotated proteins of putative or known function**
158-2040-221-2222-223-99-MK10-MK16-RNS	12	4	8
158-2040-221-2222-223-99-MK10-MK16-s0432	2	0	2
158-2040-221-2222-223-99-MK16-RNS-s0432	3	0	3
158-2040-221-2222-223-99-MK16-s0432	1	1	0
158-2040-221-2222-223-MK10-MK16-RNS-s0432	35	9	26
158-2040-221-2222-99-MK10-MK16-RNS-s0432	24	4	20
158-2040-221-2222-MK10-MK16-RNS-s0432	6	2	4
158-2040-221-223-99-MK10-MK16-RNS-s0432	5	0	5
158-2040-221-223-99-MK16-RNS-s0432	1		1
158-2040-2222-223-99-MK10-MK16-RNS-s0432	3	1	2
158-2040-2222-MK10	1	1	0
158-2040-2222-MK10-MK16-RNS-s0432	1	0	1
158-221	15	9	6
158-221-2222-223-99-MK10-MK16-RNS	3	2	1
158-221-2222-223-99-MK10-MK16-RNS-s0432	161	36	125
158-221-2222-223-99-MK10-MK16-s0432	2	0	2
158-221-2222-223-MK10-MK16-RNS-s0432	19	8	11
158-221-2222-99-MK10-MK16-RNS	1	1	0
158-221-2222-99-MK10-MK16-RNS-s0432	6	3	3
158-221-2222-99-MK16-RNS-s0432	1	1	0
158-221-2222-MK10-MK16-RNS-s0432	3	1	2
158-221-2222-MK10-RNS	2	2	0
158-221-223-99-MK10-MK16-RNS-s0432	10	3	7
158-221-223-99-MK16-RNS-s0432	1	0	1
158-221-223-99-RNS-s0432	2	0	2
158-221-223-MK10-RNS-s0432	1	0	1
158-221-223-MK16-RNS	1	1	0
158-221-99-MK10-MK16-RNS-s0432	1	1	0

158-221-MK10	1	1	0
158-221-MK10-MK16	2	2	0
158-221-RNS	4	3	1
158-221-s0432	1	1	0
158-223-MK16-RNS	1	1	0
158-223-MK16-RNS-s0432	1	0	1
158-99-MK10-MK16-RNS-s0432	1	1	0
158-RNS	5	4	1
2040-221	1	1	0
2040-221-2222-223-99-MK10-MK16-RNS-s0432	1	0	1
2040-221-2222-223-99-MK10-MK16-s0432	1	0	1
2040-221-2222-99-MK10-s0432	1	0	1
2040-2222	2	1	1
2040-2222-223-99-MK10-MK16-RNS-s0432	6	0	6
2040-2222-223-99-MK10-MK16-s0432	3	1	2
2040-223	2	2	0
2040-223-99	1	1	0
2040-223-MK16	1	0	1
2040-99	1	0	1
221-2222-223-99-MK10-MK16-s0432	1	1	0
221-2222-223-MK10-MK16-RNS-s0432	2	1	1
221-2222-99-MK10-MK16-RNS-s0432	1	1	0
221-2222-MK10	1	1	0
221-2222-MK10-MK16-RNS	1	1	0
221-RNS-s0432	1	1	0
2222-223	4	4	0
2222-223-99	2	1	1
2222-223-99-MK16-RNS-s0432	1	1	0
2222-223-MK10-MK16-RNS-s0432	1	0	1
2222-223-MK16-s0432	1	0	1
2222-223-s0432	1	0	1
2222-99	1	0	1
2222-99-MK10-MK16-s0432	1	1	0
2222-MK10	5	4	1
223-99	16	8	8
223-99-MK16	1	0	1
223-99-MK16-RNS-s0432	2	0	2
223-99-RNS-s0432	1	0	1
223-99-s0432	2	0	2
223-MK10	3	0	3
223-MK10-s0432	1	0	1
99-MK16	1	0	1
99-s0432	1	0	1
MK10-MK16	1	0	1
MK16-s0432	1	0	1

What has been found, can be concluded as: there is no accessory genome comprised of genes belonging only to the 2 of lowly virulent strains (isolated in Germany, IFB 0221 and IFB 0223) or only from highly virulent strains (isolated in Poland, IFB 0099 and IFB 0158). What is interesting, the pangenome analysis has shown that there is an accessory genome comprising ORFs from strains IFB 0158 and IFB 0221 (so strains of high and low virulence) and strains IFB 0099 and IFB 0223 (again strains of high and low virulence). The study has not revealed the accessory genome comprising ORFs from all of these 4 strains. Genes belonging to the accessorry genomes of strains IFB 0158 and IFB 221 as well as strains IFB 0099 and IFB 0223 are presented in Tables 21 and 22. For accessory genome of IFB 0158-IFB 0221, 9 hypothetical proteins have been found, for accessory genome of IFB 0099-IFB 0223, 8 hypothetical proteins have been found and they were excluded from Tables 21 and 22.

Table 21.Accessory genomes of strains IFB 0158-IFB 0221.

Accessory genome	Gene name	Product
IFB 0158 - IFB 0221	*trg_14;trg_16*	Ribose and galactose chemoreceptor protein
IFB 0158 - IFB 0221	*pehX_2;pehX_3*	Exo-poly-alpha-D-galacturonosidase precursor
IFB 0158 - IFB 0221	*hcpA_3*	Secreted protein hcp
IFB 0158 - IFB 0221	*pehX_1*	Exo-poly-alpha-D-galacturonosidase precursor
IFB 0158 - IFB 0221	*hcpA_6;hcpA_1*	Secreted protein hcp
IFB 0158 - IFB 0221	*tar_5;tar_2*	Aspartate chemoreceptor protein

Table 22. Accessory genomes of strains IFB 0099-IFB 0223.

Accessory genome	Gene name	Product
IFB 0099- IFB 0223	*pksN_2;pksR_1*	Polyketide synthase PksN;Polyketide synthase PksR
IFB 0099- IFB 0223	*pksN_3*	Polyketide synthase PksN
IFB 0099- IFB 0223		beta-methylgalactoside transporter inner membrane component
IFB 0099- IFB 0223	*pksJ_3*	Polyketide synthase PksJ
IFB 0099- IFB 0223		H+ Antiporter protein
IFB 0099- IFB 0223	*pksN_5;pksJ_1*	Polyketide synthase PksN;Polyketide synthase PksJ
IFB 0099- IFB 0223		putative ATP-binding protein involved in virulence
IFB 0099- IFB 0223	*nifL_1*	Nitrogen fixation regulatory protein

What is interesting the accessory genome of strains IFB 0158 and IFB 0221 includes genes encoding pectinolytic enzymes such as *pehX*and *hcp*geneswhich encode Hcp- T6SS effectors. On the other hand the accessory genome of IFB 0099 and IFB 0223 includes (additionally to core genome) genes encoding polyketide sythases.

In Table 23, there are presented the Average Nucleotide Identity (ANI) values for strains representing high and low virulence (IFB 0099 and IFB 0223, respectively) in comparison to *D. dadantii* 3937. What is also interesting strains where one is highly virulent (IFB 0099) and the other is of low virulence (IFB 0223) differ in average nucleotide identity by only 0.02 %.

Table 23. Average nucleotide identity (ANI) of highly and lowly virulent *D. solani* strains (IFB 0099 and IFB 0223). Only lower diagonal values are reported

	*D. solani*IFB0099	*D. solani*IFB0223	*Dickeya dadantii* 3937
*D. solani*IFB0099	--		
*D. solani*IFB0223	99.98 %	--	
Dickeya dadantii 3937	94.04 %	94.02 %	--

The number of unique ORFs is 823 (the unique genome of *D. solani*), so on average each strain possesses *circa* 82 unique ORFs. In reality, it does not look like that. Table 24 presents the contents of unique genome for each strain and it is clear that the numbers differ among strains. Strain IFB 0099 possesses the biggest number, 271 ORFs, of unique ORFs (this strain has always indicated high virulence). On the other hand, the strain IFB 0223 (of low virulence) has 198 unique ORFs. Interestingly, two other strains that were assigned as highly and lowly virulent (IFB 0158 and IFB 221, respectively) possess significantly fewer unique ORFs (13 and 23 respectively). Among the unique genome there are assigned hypothetical proteins as well as proteins of a putative or known function. *D. solani* strain D s0432-1 possesses 16 unique ORFs of which all are hypothetical proteins of an unknown function. Strain IFB 0158 possesses 12 (out of 13) hypothetical proteins sequences.

Table 24. Unique genome composition of the *D. solani* pangenome.

Genome	Number of unique ORFs	Number of hypothetical proteins	Number of annotated proteins of putative or known function
IFB0099	271	69	202
IFB 0158	13	12	1
IFB0223	198	77	121
IFB 0221	23	19	4
IPO2222 TS	63	41	23
GBBC2040	112	102	10
MK10	103	18	85
MK16	12	6	6
D s0432-1	16	16	0
RNS 08.23.3.1A (IPO 3337)	12	11	1

7.3.4 Analysis of the presence and homology level of pathogenicity-related genes

The main virulence factors of SRE are already mentioned PCWDE, especially pectinolytic enzymes. That is why we performed LS-BSR analysis that allowed us to state if all the genes described in *D. dadantii* 3937 are present in each of the analyzed genomes and that would show the level of their identity. LS-BSR method allows a comparison of a large number of genomes. It translates the nucleotide sequence into protein sequence and then allows searching for orthologous proteins.

The presence of pathogenicity-related genes (5 major pectate lyases PelA, PelB, PelC, PelD and PelE; 3 secondary pectate lyases – PelI, PelL, PelZ and one cellulase CelZ) in the genomes of 10 *D. solani* strains was checked by LS-BSR search using the protein sequences described for *D. dadantii* 3937 as queries. All the mentioned proteins appear to have anortholog inside each strain, with a few exceptions (such as *pelL* in strains IFB 0099, IFB 0223 and GBBC 2040 has a low LS-BSR score, which could indicate a low sequence similarity). The result of this analysis is presented in Figure 44.

What is interesting when a Local BLAST (implemented in BioEdit) was performed using a *pelL* gene from *D. dadantii* 3937 as query against IFB 0099, IFB 0223 and GBBC 2040, only the last exhibited a shorter nucleotide sequence of the *pelL* gene (207 nucleotides shorter sequence). Strains IFB 0099 and IFB 0223 possess full *pelL* gene sequence and it has 92 % identity to *D. dadantii* 3937 *pelL* gene. The ambiguous LS-BSR result for IFB 0099 and IFB

0223 can be explained as a problem in translating the nucleotide sequence into protein sequence by the program. In the case of GBBC 2040, the cause of a shorter sequence can be the sequencing problem and/or the sequence can be truncated because of the existence of a gap in that place.

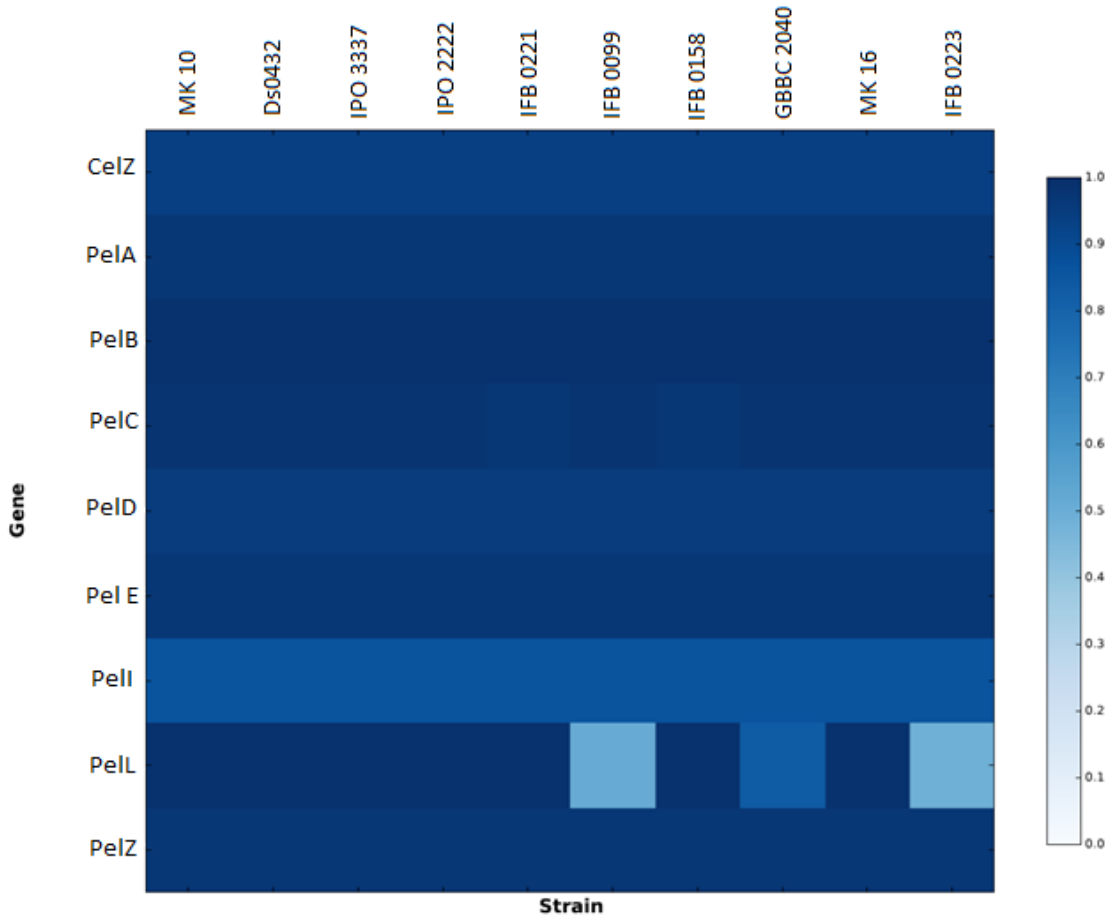

Figure 44. LS-BSR analysis on the 9 cell wall-degrading enzymes protein sequence. Color intensity is proportional to the large-scale blast score ratio; the higher, the higher the probability that the query gene is present in the target genome.

7.3.5 Analysis of presence and homology level of regulators of pathogenicity-related genes

A similar analysis to the one performed on PCWDE has been conducted on the sequences of 19 regulators genes coding for proteins involved in regulation of the expression of virulence factors genes. All the regulatory proteins appear to have an ortholog inside each strain, with a few exceptions (such as *expI* in strains MK 10 and IFB 0099, *expR* in strains

GBBC 2040 and IFB 0223, and *vfmE* in strain IFB 0099 that had a low LS-BSR score, which could indicate a low sequence similarity). The result of this analysis is presented in Figure 45. Again, the ambigous results from LS-BSR analysis were confirmed by Local BLAST using the nucleotide sequences from *D. dadantii* 3937 against the selected genomes. The Local BLAST of *D. dadantii* 3937 *expI* nucleotide sequence against MK 10 genome has proved an existence of a full sequence of *expI* gene in its genome (and it is in 94 % identitical). Strain IFB 0099 also possesses a full sequence of *expI* in its genome at 94 % of identity (based on local blast using *expI* nucleotide sequence from *D. dadantii* 3937 as query). Local blast of *expR* nucleotide sequence from *D. dadantii* 3937 against the genome of strain GBBC 2040 showed that this sequence is truncated (179 nucleotides). At the same time, this sequence is not truncated in the IFB 0223 genome (and it has 95 % of identities). The Local BLAST was also performed using a *vmfE* nucleotide sequence from *D. dadantii* 3937 against IFB 0099 genome and again it was proved that the sequence is present in full length in the IFB 0099 genome (and it is 95 % identical). Again, the ambiguous LS-BSR result could be explained as a problem in translating the nucleotide sequence into protein sequence by the program. In the case of GBBC 2040, the cause of a shorter sequence can be a sequencing problem and/or the sequence can be truncated because of the existence of a gap in that place.

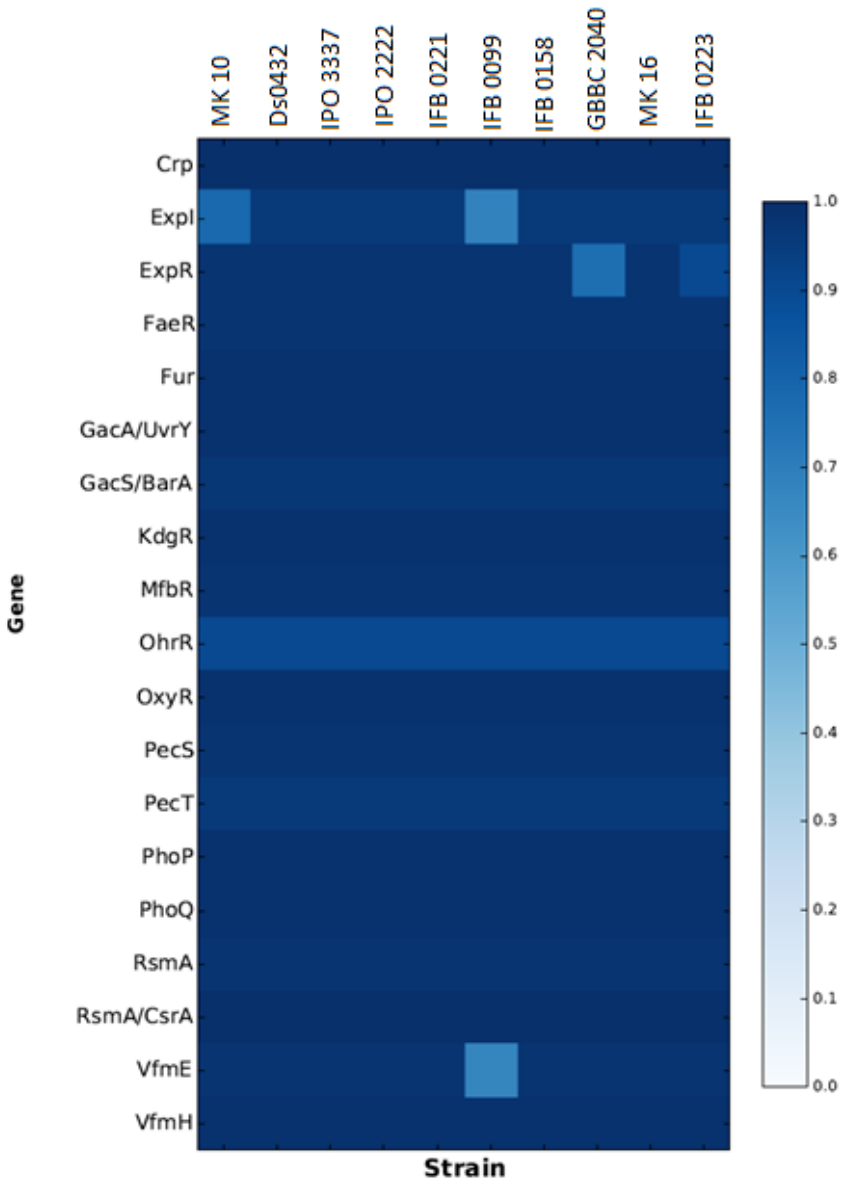

Figure 45. LS-BSR analysis on the regulator proteins genes. Color intensity is proportional to the large-scale blast score ratio; the higher, the higher the probability that the query gene is present in the target genome.

7.3.6 Phylogeny assessment

Phylogenetic analysis was performed on the sequences of genes belonging to the core genome of *D. solani* pangenome. The core Ortologous Groups (OGs) have been used to build a core concatemer of 3 416 nucleotide alignments, using those genes whose nucleotide length difference was below 60 bp. Each OG has been aligned using Muscle (version 3.8.31) and the resulting alignments have been concatenated and converted to the NEXUS format. The phylogenetic tree has been constructed using MrBayes (version 3.2.2). The tree has been manipulated using FigTree (version 1.4.0). Strain IFB 0099 appears as the most diverse, as

compared to the others; also strain RNS08.23.3.1A (IPO3337) shows a high degree of differences (Figure 46). The scale presented in the Figure shows the nucleotide substitution rate in the studied genomes (2.0 E^{-5}which means substitution rate is present in a number of substituted nucleotides per 10 000 bp). That means that strain IFB 0099 has the highest nucleotide substitution rate and might be evolving the fastest out of the studied strains (because its core genome has the highest number of substitutions per 10 000 bp).

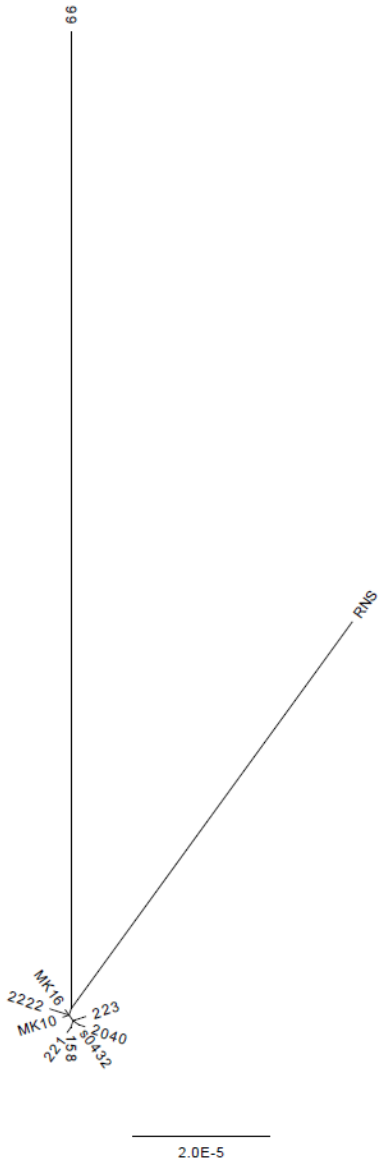

Figure 46. Phylogenetic tree of the ten *D. solanii* strains. The phylogenetic tree has been constructed using MrBayes (version 3.2.2). In description shortcuts of strains used as follows: 2040 – strain GBBC 2040; s0432 – strain ; 158 – IFB 0158, 221 – strain IFB 0221; MK 10 – strain MK 10; 2222 – strain IPO 2222; MK 16 – strain MK 16; 99 – strain IFB 0099; RNS – strain RNS 08.23.3.1A;, 223 – strain IFB 0223.

In conclusion: 10 *D. solani* genomes are structurally similar (with only a few inversions and translocations among all 10 tested genomes). They all possess selected for analysis plant cell-wall degrading enzymes and they are highly homologous to *D. dadantii* 3937 and identical among *D. solani* strains. They all possess 19 selected proteins involved in the regulation of genes involved in pathogenicity and, again, they are highly homologous to *D. dadantii* 3937 and identical among themselves. The phylogeny analysis based on core genome alignemnts shows that two strains: IFB 0099 and RNS 08.23.3.1A (IPO 3337) have higher substitution rates than other 8 strains suggesting that they might evolve faster than others. The strains of high and low virulence (IFB 0099 and IFB 0223) are identical in 99.98 % according to ANI values. The LS-BSR analysis of the 10 tested genomes did not distinguish differences between strains in the sequences of 9 PCWDE and their regulators. Strains differ in the accessory and unique genes reperotire. *D solani* strains possess T6SS effector proteins as well as polyketide synthases.

8 Discussion

The emergence of a new, highly virulent pathogenic SRE species which *D. solani* is has become a big problem for the producers of seed potatoes as well as for the farmers. The presence of this pathogen raised a big threat to potato growers all around Europe and North-African countries. At the same time, there are no methods of curing the disease and all of the protection is based on preventory methods, for example potato seed certification. Very important is testing seed potatoes for the presence of SRE before selling and exporting them. For example for high-grade seed tubers, there is in some countries there is zero tolerance for blackleg and soft rot diseases in both field and tuber inspections (Toth et al., 2011).

However, the knowledge about *D. solani* biology is limited. Its fast spreading across Europe and Northern African countries is still not fully understood, but researchers suspect climate change as well as international exchange of certified material of seed potatoes. Tsror and colleagues (2013) underline that the import of seed potatoes from European countries, such as the Netherlands or Germany, could be the cause of *D. solani*'s emergence in Israeli potato plantations.

Little is known about the biology, virulence mechanisms and epidemiology of *D. solani*. Genomic analysis of *D. solani* has stated that this species is mostly related to *D. dadantii* 3937 strain (94 % ANI), (Garlant et al. 2013; Pedron et al. 2014; Van der Wolf et al. 2014). It is fortunate, because this strain appears to be the most studied. A multitude of molecular techniques to study *D. dadantii* 3937, have been developed. Potrykus and colleagues (2014) adapted the techniques used for *D. dadantii* 3937 mutagenesis and applied them in the *D. solani* study, where the functions of three main pathogenicity regulators were studied. It confirmed that not only the *in silico* genomic study can be successfully implemented in the research dedicated to the study of pathogenicity mechanism in *D. solani*.

This work presents a complex phenotypic and genotypic analysis of 15 *D. solani* strains originating from different climatic conditions. It also contains a study of potato tuber extract influence on pectinolytic activity of *D. solani* as well as its influence on the expression of selected genes of *D. solani* in comparison to *D. dadantii* 3937. The last part of this work is devoted to comparative genomic analyses of 10 *D. solani* genomic sequences.

8.1 *D. solani* strains indicated phenotypic variabilities apart from their genotypic homogeneity

Analysis of several phenotypic features of the range of *D. solani* strains originating from three countries with different climates: Poland, Finland and Israel indicated differences in PCWDE production, swimming and swarming ability and in an ability to macerate potato tissue. Polish strains indicated the lowest variabilities and the highest value of pectinolytic, cellulolytic and proteolytic activities at 18, 28 and 37 °C. They also have the highest total pectate lyases activity and the ability to macerate potato tissue regardless of the inoculum level. Polish and Finnish strains have a better swimming ability than Israeli strains.

The presented study also revealed for the first time that *D. solani* has higher pectinolytic, cellulolytic and proteolytic activities than *D. dianthicola* and *D. dadantii* at almost all tested temperatures and the highest total pectate lyases activity at 28 °C (Table 13). This could explain the fact that strains of *D. solani* are better fitted to induce diseases such as blackleg and soft-rot than *D. dianthicola*. Czajkowski and colleagues (2012) compared *D. solani* strains to *D. dianthicola* strains and showed that *D. solani* possesses features which allow more efficient potato plant colonization than *D. dianthicola*especially at28 °C. It suggested that *D. solani* could be a stronger competitor in the potato ecosystem. In greenhouse experiments performed at 28 °C, roots weremore rapidly colonized by *D. solani* than by *D. dianthicola* and 30 days after inoculation higher densities of *D. solani*were found in stolons and progeny tubers. In tubers co-inoculated by vacuum infiltration with GFP-tagged *D. solani* and DsRed-tagged *D. dianthicola, D. solani* strain outcompeted *D. dianthicola*. Moreover Czajkowski and colleagues (2012) compared the susceptibility of *D. solani* and *D. dianthicola* to 363 strains of saprophytic bacteria, potential biocontrol agents, including species such as: *Serratia plymuthica, Pseudomonas spp., Delftia acidovorans, Pantoea agglomerans, D. acidovorans, Rahnella* sp., *Klebsiella* sp. and *Acinetobacter* sp., isolated from tuber homogenates. *D. dianthicola* was susceptible to 80% of saprophytic bacteria strains, while *D. solani* was susceptible to only 31 % of the tested strains. The work of Tsror and colleagues (2013) has shown that bacteria from both species: *D. solani* and *D. dadantii* subs. *dieffenbachiae* have a higher pectinolytic activity than *D. dianthicola*.

The presented thesis tried to answer the question if the *D. solani* strains originating from different climatic conditions differ and if they have better fitness to induce disease symptoms than strains from other species and if that can be explained by the influence of origin (Poland,

Finland and Israel) or growth temperature. In Poland and Finland the climate is cold and moderate (according to Koeppen described in Pidwirny et al., 2006) and it only differs in regard to the summer, which in Poland is moderate and in Finland is short and cold. How does it relate to the climate in Israel which is hot moderate, steppe with hot summer?

Works of Degefu and colleagues (2013) and Potrykus and colleagues (in press 2015) stated that there is an influence of climate change in terms of *D. solani* outbreaks in Finland and Poland. They suggested that the prevalence of *Dickeya* spp. is higher when the summer is hotter and dry. The climate change in the years 1976 – 2006 (Figure 47) shows that the mean temperature values increased in the range of 0.4 to 1.0 °C per decade in Poland and Finland, respectively. The prediction of annual temperature increase in Europe is between 0.3 and 0.35 °C for Poland and Finland respectively, according to the European Environmental Agency.

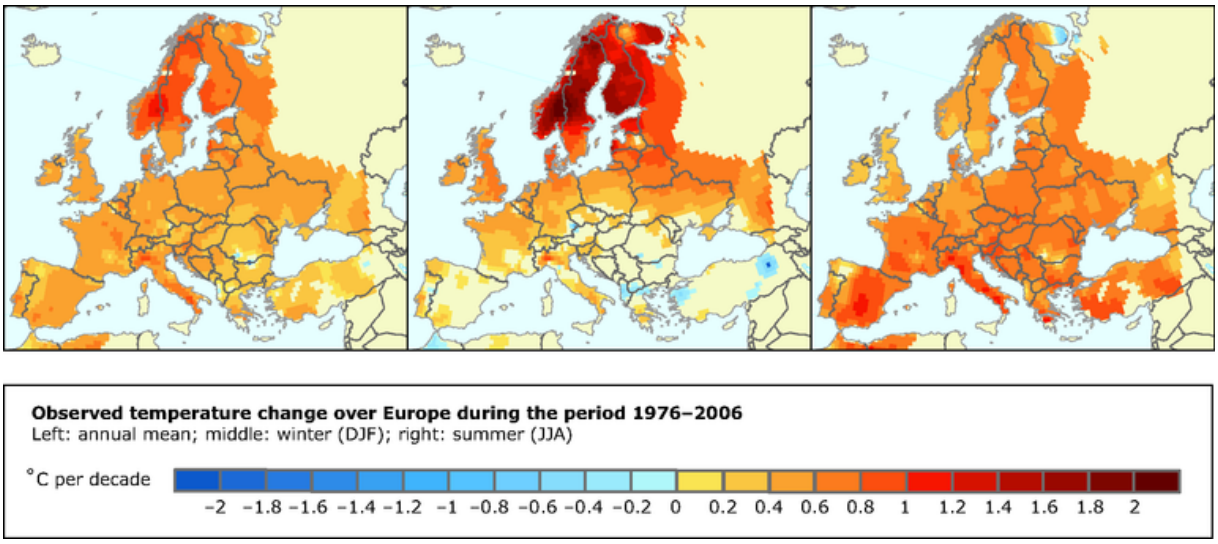

Figure 47. Climate change in Europe in years 1976-2006. The European Environment Agency (EEA) (http://www.eea.europa.eu/data-and-maps/figures/observed-temperature-change-over-europe-1976-2006/map-5-1-climate-change-2008-observed-temperature-change.eps)

The data presented in this thesis indicated a higher impact of the species of bacteria on the level of expression of the genes coding CWDE; *D. solani* indicated better abilities to cause disease symptoms than other tested *Dickeya* species. This could explain that if different *Dickeya* species are present in the same ecological niche, *D. solani* has a higher chance to cause disease outbreak.

Tsror and colleagues (2013) stated that *D. solani* strains isolated in Israel are more virulent than those isolated in Europe. My study indicated the opposite. How can that be explained? First of all, in the Tsrors study, 22 Israeli strains of *D. solani* were analyzed, here only 5. They only used one strain isolated in Poland, namely IFB 0099 (IPO2276), and a few strains isolated in the Netherlands, but none from Finland. On the other hand Israeli strains analyzed by Tsror and colleagues (2013) have also been used in my study. In the presented study, four out of five tested strains had a low pectate lyase activity in induced conditions. The difference could be explained by different methodology for pectate lyase activity estimation. In my study, bacteria were grown on minimal medium M63 Y supplemented with PGA as an inducer of the expression of genes coding pectinolytic enzymes, while in Tsror work pectate lyases activity was estimated for the bacteria isolated from macerated potato tubers. The study of the role of different pectate lyases in *D. dadantii* 3937 (reviewed by Hugouvieux-Cotte-Patat et al., 2014) has shown that different pectate lyases are differently induced. Pectate lyases are divided into the main and secondary pectate lyases. As presented here, the main pectate lyase *pelD* is mainly induced by PGA, while secondary pectate lyase *pelL* is induced by both, PGA and the plant extract. That might be a point that could explain the high total pectate lyases activity (major and secondary Pels) of Israeli strains, which also indicated high levels of *pelL* gene expression in some of the tested strains. The secondary pectate lyases can play important role in pathogenicity of *D. solani*; but more studies are necessary to determine.

The hereby presented data indicated that the species of bacteria has a higher impact on the production of PCWDE than the temperature in which bacteria are growing, even though both effects are significantly important for the exhibited traits. That is interesting and could explain why *D. solani* is a bigger threat to potato plantations than *D. dadantii* or *D. dianthicola* in moderate climates. The impact of the strains origin (different climate) was less important than the temperature of incubation when the origin and the temperature were considered in terms of their influence on the expression of genes coding PCWDE of *D. solani* strains.

The PCWDE enzyme production is sensitive to plenty of environmental factors. Hugouvieux-Cotte-Pattat and colleagues (1996) sums up that enzyme synthesis was very sensitive to growth phase, nitrogen starvation, anaerobiosis, growth temperature, osmolarity of the growth medium, and presence of a readily utilizable carbon source. The effect of growth conditions on the increase of *pel* genes expression (for instance, anaerobiosis and low temperature) was more visible in the absence of PGA (inducing factor), since the responses to these different inducing conditions were not synergistic.

What is interesting, even though there are significant differences in phenotypic traits exhibited by *D. solani* strains originating from different climatic conditions, there are no differences between those strains in molecular fingerprinting performed with rep-PCR and RFLP-PFGE methods. Finally, it does not matter where they were isolated, the molecular patterns are the same. These results are in agreement with the results of Degefu and colleagues (2013) and Wolf et al (2014) which also indicated high similarities between *D. solani* strains.

The variable tandem repeats method applied by Parkinson et al. 2015 has also revealed low variability among 54 tested *D. solani* strains. What is more 4 out of 16 of *D. solani*strains tested in my thesis (Polish IFB 0099, Israeli IFB 0124 and IFB 0125 and IFB 0123) have been analyzed by Parkinson and colleagues (2015). They discovered that *D. solani*^{TS} (IFB 0123) has a different VNTR profile than 3 other above- mentioned strains. But the difference is only in one locus out of 5 tested. Parkinson and colleagues (2015) stated that the limited variation in VNTR profiles of *D. solani* may reflect a limited number of *D. solani* introductions and/or the short time period since the pathogen's first emergence in Europe. In the same study, 19 VNTR profiles for 45 *D. dianthicola* strains have been distinguished (Parkinson et al., 2015) which means that *D. dianthicola* is much more variable than *D. solani*.

To sum up, *D. solani* strains are better fit to induce disease symptoms on potatoes. Strains of this species are homogenous genetically, but they differ phenotypically, especially in different temperature conditions. Polish strains seem to be more virulent than strains from Finland and Israel regardless of the tested conditions.

8.2 Influence of plant tissue components on the expression of genes involved in pathogenesis

The best studied plant-derived inducers of the expression of genes coding Pels are pectin derivatives (Hugouvieux- Cotte-Pattat and Robert-Baudouy 1989; Roy et al. 1999; Blot et al. 2002). However, pectin derivatives are unlikely to be the sole host derived inducers of PCWDEs synthesis because plant metabolites other than pectin were also shown to stimulate Pel's production (Bourson et al. 1993; Kelemu and Collmer 1993; Brencic and Winans 2005). Unfortunately, those metabolites were poorly characterized.

The data presented in this thesis indicated that the expression of *pelL* and *lfaA* genes was induced by crude extract from potato tuber. *pelL* gene expression was also induced by PGA, while *lfaA* expression was induced by plant extract exclusively. The crude potato tuber extract did induce the pectate lyase activity of *D. solani* strains, but not so highly as PGA.

Van Gijsegem and collegues (2008) studied the influence of plant extract on gene expression of several genes being under regulation of LacI family proteins and found 2 genes extremely induced by plant extract. They studied the influence of several compounds known to be present in plant tissue, such as: arabinogalactan, L-arabinose, arbutine, cellobiose, D-fructose, L-fucose, D-galactose, D-galacturonate, D-glucose, D-glucuronate, D-mannose, pectin, polygalacturonate, L-rhamnose, rham- nogalacturonan, salicine, sucrose, or D-xylose and none of them exhibited the inducing impact. Wlodarczyk (2010) fractioned crude potato tubers extract and studied the influence of its fractions on *lfaA* gene expression. It seems that several inducer molecules are present in potato tubers extracts, because several fractions were able to induce gene expression, but the pure active compound/s were not characterized.

The induction of expression of PCWDE genes in *P. atrosepticum* in the presence of potato stem or tuber water extracts was demonstrated by Mattinen et al. (2008) by means of microarray analysis. In that study the inducers were shown to be of low molecular weight (>5 kDa compounds). In the study of Tarasova and colleagues (2013) it was shown that the compounds present in the potato extract enhance extracellular pectate lyase activity in *P. atrosepticum* SCRI1043. These compounds were found to be less than 1 kDa, nonproteinaceous, hydrophilic, slightly negatively charged molecules. The possibility of their action through quorum sensing- and KdgR-mediated pathways was analyzed.

Tu sum up: *pelL* and *lfaA* genes of *D. solani* were found to be induced by potato tuber extract, but the inducing compound/s still has/have to be elucidated as well as its influence on *D. solani* virulence.

8.3 Study of *D. solani* pangenome

Advances in the sequencing of complete genomes, growth of data deposited in databases and development of computer programs for gene identification and protein similarity analysis are powerful tools. They make it possible to reconstitute a major fraction of

metabolism of a bacterium given its newly sequenced genome. Genome sequences are also enabling determination of phylogenetic relationships among species based upon sequences for multiple proteins.

Genomes of 10 *D. solani* strains were compared and indicated similar structure. The core genome includes all of the housekeeping genes as well as the genes involved in pathogenicity development;genes coding virulence factors like pectinolytic, cellulolytic and proteolytic enzymes and their regulators as well as several genes encoding polyketide synthases (PKS) and T6SS effectors. The analysis of protein sequences of 9 PCWDE and 19 of pathogenicity regulators revealed that they are 100 % identical among 10 tested *D. solani* strains. The analyzed strains differ in numbers of ORFs in accessory and unique genomes. Strains of high and low virulence share accessory genomes, but accessory genome of only highly or only lowly virulent strains was not determined.

Garlant and colleagues (2013) were the first to describe the presence ofgene clusters encoding PKS's in the *D. solani* genome and stated that these clusters are missing in *D. dadantii* 3937 genome (Figure 48). They compared *D. solani* D s0432-1 genome to the genomes of 9 *Dickeya* and *Pectobacterium* strains to identify similarities and differences between *D. solani* and other sequenced *Dickeya* and *Pectobacterium* strains. It was evident that *D. solani* strain D s0432-1 shares large parts of its genome with *D. dadantii* 3937 and other *Dickeya* spp.strains, but some parts of the D s0432-1 genome have a similar sequence only in one or a few *Dickeya* strains (Figure 48). They described regions specific for *D. solani*, coding for non-ribosomal peptide synthetases (NRPS) and PKS (1, 2 and 3 in Figure 48). PKS gene clusters may be involved in the production of toxic secondary metabolites, such as oocydin and zeamine. They also mentioned that the effectors of T6SS, hemolysin-coregulated proteins (Hcp) were found in *D. solani* genome. Horizontal gene transfer is observed throughout bacteria, with implications for the acquisition of novel biological functions. Pedron and colleagues (2014) also found NRPS/PKS encoding genes and T5SS and T6SS-related toxin-antitoxin systems in *D. solani* IPO 3337 genome and mentioned they are present in another *D. solani* draft genomes available at that time in the databases (IPO2222, MK10, MK16 and GBBC 2040). After adding to the analysis another 4 *D. solani* genomes sequenced in our Laboratory (IFB 0099, IFB 0158, IFB 0221 and IFB 0223) I can state that they are in the core genome of *D. solani*. These genes may contribute to bacteria-bacteria interactions and to the fitness of *D. solani*, its advantages in different ecological niches and its high ability to cause disease symptoms.

The NCBI database provides a description of available *D. solani* genomic sequences. It also presents the phylogenetic dendrogram made on the basis of genomic BLAST of 7 available *D. solani* genomes (Figure 49). This also includes the highly virulent *D. solani* IFB 0099 strain genome sequenced in our Laboratory (Golanowska et al., 2015). The structure of this tree confirms our phylogenetic analysis, showing that *D. solani* IFB 0099 strain, isolated in Poland, is the most distant from other strains. Our study suggests faster evolution of this strain.

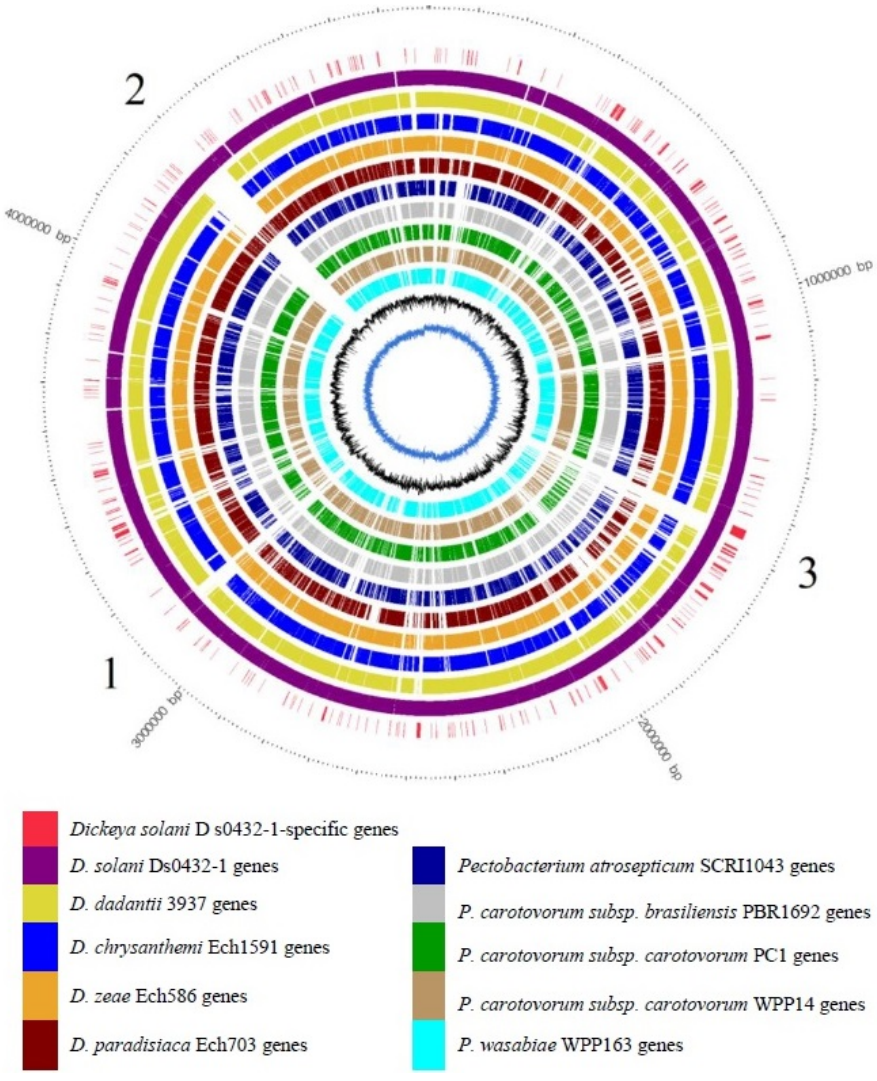

Figure 48. Comparative genomics between *Dickeya* and *Pectobacterium* strains. Comparison of gene coding sequences between *D. solani* D s0432-1 and other sequenced *Dickeya* and *Pectobacterium* spp. strains showing three large genomic regions (1, 2 and 3) coding for non-ribosomal peptide synthetases and polyketide synthetases (numbered according to the order of description in the results). The tentative *D. solani*-specific open reading frames are shown as the outermost ring. Garlant et al., 2013.

Figure 49. Dendrogram based on 7 *D. solani* genomes done by the use of genomic BLAST. NCBI, July 2015.

The presented study covering the comparative analysis of 10 *D. solani* strains confirms that *D. solani* strains show a low level of variability. The already mentioned analysis of VNTRs by Parkinson and colleagues 2015 has implicated that the limited variation in VNTR profiles of *D. solani* may reflect a limited number of *D. solani* introductions and/or the short time period since the pathogen's first emergence in Europe.

What is interesting, Naushad and colleagues (2013) performed a comparative analysis of protein sequences from the *Dickeya*, *Pectobacterium* and *Brenneria* genomes and identified 10 conserved signature indels (CSI) and 5 conserved signature proteins (CSP) that are either uniquely or largely found in all genome-sequenced species from these genera, but not present in any other bacteria in the database. In addition, they identified 10 CSIs and 17 CSPs that are specifically present in either all or most sequenced *Dickeya* species/strains, and 6 CSIs and 19 CSPs that are uniquely found in the sequenced *Pectobacterium* genomes. They also found 3 CSIs and 1 CSP that are specifically shared by members of the genera *Pectobacterium* and *Brenneria*, but absent in species of the genus *Dickeya*, indicating that the former two genera shared a common ancestor exclusive of *Dickeya*.

To sum up: core genome of *D. solani* contained genes coding main virulence factors and genes coding their regulators; in addition, these genes indicated 100% homology in 10 analyzed strains. *D. solani* strains form a very homogenous group, whose core genome contains at least two, important for competition as well as pathogenicity, sets of virulence factors, such as NRSP/PKS and T6SS effectors, in addition to well known plant cell-wall degrading enzymes.

9 Conclusions

1. *Dickeya solani* strains are homogenous genetically, but indicate some phenotypic differences.

2. *D. solani* strains have higher plant cell-wall degrading enzymes activities (such as pectinases, cellulases and proteases) than *D. dianthicola* strains, regardless of the temperature of incubation.

3. Polish *D. solani* strains are superior to strains originating from Finland and Israel in the production of PCWDE and ability to macerate potato tissue.

4. Potato tuber extract induces the expression of *pelL* (secondary pectate lyase) and *lfaA* genes, but not *pelD* (major pectate lyase) and *tssK*.

5. Core genome of *D. solani* comprises genes encodingmajor virulence factors (PCWDE) and their regulators and also genes coding NRSP/PKSand T6SS effectors.

6. Phylogenetic analysis performed on the basis of the core genomes of *D. solani* indicated that highly virulent Polish *D. solani* strain – IFB 0099 is more distant phylogenetically.

10 Bibliography

Agrios, G. N. (2005). Plant Pathology. 5th eds.

Andro, T., Chambost, J. P., Kotoujansky, A., Cattaneo, J., Bertheau, Y., Barras, F., Coleno, A. (1984). Mutants of *Erwinia chrysanthemi* defective in secretion of pectinase and cellulase. Journal of Bacteriology, 160(3), 1199-1203.

Antunez-Lamas M, Cabrera-Ordonez E, Lopez-Solanilla E, Raposo R, Trelles-Salazar O, et al. (2009a). Role of motility and chemotaxis in the pathogenesis of *Dickeya dadantii* 3937 (ex Erwinia chrysanthemi 3937). Microbiology 155:434–42

Antunez-Lamas M, Cabrera E, L´opez-Solanilla E, Solano R,Gonz´alez-Melendi P, et al. (2009b). Bacterial chemoattraction towards jasmonate plays a role in the entry of *Dickeya dadantii* through wounded tissues. Mol. Microbiol. 74:662–71

Andersson R.A., Eriksson A.R., Heikinheimo R., Mäe A., Pirhonen M., Kõiv V., Hyytiäinen H., Tuikkala A., Palva E.T. (2000) Quorum sensing in the plant pathogen *Erwinia carotovora* subsp. carotovora:the role of expR(Ecc). Molecular Plant–Microbe Interactions, 13, 384–393

Aoki SK, Diner EJ, de Roodenbeke C, Burgess BR, Poole SJ, et al. 2010. A widespread family of polymorphic contact-dependent toxin delivery systems in bacteria. Nature 468:439–42

Bardonnet, N., and Blanco, C. (1992) uidA antibiotic resistance cassettes for insertion mutagenesis, gene fusion and genetic constructions. FEMS Microbiol Lett 93: 243-248

Barras F, Thurn KK, Chatterjee AK. 1987. Resolution of four pectate lyase structural genes of *Erwinia chrysanthemi* (EC16) and characterization of the enzymes produced in Escherichia coli. Mol. Gen. Genet. 209:319–25

Barras F, Van Gijsegem F, Chatterjee AK. 1994. Extracellular enzymes and pathogenesis of soft-rot *Erwinia*. Annu. Rev. Phytopathol. 32:201–34

Bell KS, Sebaihia M, Pritchard L, Holden MTG, Hyman LJ, Holeva MC, Thomson NR, Bentley SD, Churcher LJC, Mungall K et al. 2004 : Genome sequence of the enterobacterial phytopathogen *Erwinia carotovora* subsp. atroseptica and characterization of virulence factors. Proc Natl Acad Sci USA, 101:11105-11110.

Bertheau Y, Madgidi-Hervan E, Kotou- janskyA, Nguyen-The C, Andro T, et al. 1984. Detection of depolymerase isoen- zymes after electrophoresis or electro- focusing, or in titration curves. AnalBiochem. 139:383–89

Bhandari, V., Naushad, H. S. & Gupta, R. S. (2012). Protein based molecular markers provide reliable means to understand prokaryotic phylogeny and support Darwinian mode of evolution. Front Cell Infect Microbiol 2, 98.

Blot, N., Berrier, C., Hugouvieux-Cotte-Pattat, N., Ghazi, A., & Condemine, G. (2002). The oligogalacturonate-specific porin KdgM of *Erwinia chrysanthemi* belongs to a new porin family. Journal of Biological Chemistry, 277(10), 7936-7944.

Boccara, M., Aymeric, J. L., & Camus, C. (1994). Role of endoglucanases in *Erwinia chrysanthemi* 3937 virulence on *Saintpaulia ionantha*. Journal of bacteriology, 176(5), 1524-1526.

Bourson, C., S. Favey, S. Reverchon, & J. Robert-Baudouy. (1993). Regulation of the expression of a pelA::uidA fusion in *Erwinia chrysanthemi* and demonstration of the synergistic action of plant extract with polygalacturonate on pectate lyase synthesis.Journal of General Microbiology139: 1-9

Boyer, M.-H., J.-P. Chambost, M. Magnan, and J. Cattaneo. 1984.Carboxymethyl-cellulase from *Erwinia chrysanthemi*. I. Productionand regulation of extracellular carboxy-methylcellulase. J. Biotechnol.1:229-239.

Brady, C. L., Cleenwerck, I., Denman, S., Venter, S. N., Rodriguez-Palenzuela, P., Coutinho, T. A. & De Vos, P. (2012). Proposal to reclassify *Brenneria quercina* (Hildebrand and Schroth 1967) Hauben et al. 1999 into a new , *Lonsdalea* gen. nov., as *Lonsdalea quercina* comb. nov., descriptions of *Lonsdalea quercina* subsp. quercina comb. nov., *Lonsdalea quercina* subsp. iberica subsp. nov. and *Lonsdalea quercina* subsp. britannica subsp. nov., emendation of the description of the genus *Brenneria,* reclassification of *Dickeya dieffenbachiae* as *Dickeya dadantii* subsp. *dieffenbachiae* comb. nov., and emendation of the description of *Dickeya dadantii*. International Journal of Systematic and Evolutionary Microbiology62, 1592-1602.

Brencic, A., & Winans, S. C. (2005). Detection of and response to signals involved in host-microbe interactions by plant-associated bacteria. Microbiology and Molecular Biology Reviews, 69(1), 155-194.

Brierley, J., Lees, A., Hilton, A., Wale, S., Peters, J., Elphinstone, J., & Boonham, N. (2008). Improving decision making for the management of potato diseases using real-time diagnostics. Potato Council Final Report, 253, 2008.

Burkholder, W. H., MacFadden, L. H., Dimock, A. H. (1953). A bacterial blight of chrysanthemums. Phytopathology 43: 522–525.

Castillo, A., & Reverchon, S. (1997). Characterization of the pecT control region from Erwinia chrysanthemi 3937. Journal of bacteriology, 179(15), 4909-4918.

Charkowsky A, (2009) Decaying signals: will understanding bacterial-plant communications lead to control of soft rot. Curr Opin Biotechnol 20: 1–7.

Charkowski A, Blanco C, Condemine G, Expert D, Franza T, Hayes C, Hugouvieux-Cotte-Pattat N, López Solanilla E, Low D, Moleleki L, Pirhonen M, Pitman A, Perna N, Reverchon S, Rodríguez Palenzuela P, San Francisco M, Toth I, Tsuyumu S, van der Waals J, van der Wolf J, Van Gijsegem F, Yang CH, Yedidia I. (2012) The role of secretion systems and small molecules in soft-rot enterobacteriaceae pathogenicity. Annual Review of Phytopatholy.50:425-449.

Chen, X. F., Zhang, H. L., & Chen, J. (2015). First report of *Dickeya solani* causing soft rot in imported bulbs of Hyacinthus orientalis in China. Plant Disease, 99(1), 155-155.

Chesnokova, O., Coutinho, J. B., Khan, I. H., Mikhail, M. S., & Kado, C. I. (1997). Characterization of flagella genes of *Agrobacterium tumefaciens*, and the effect of a bald strain on virulence. Molecular microbiology, 23(3), 579-590.

Christie PJ, Cascales E: Structural and dynamic properties of bacterial type IV secretion systems (review). Mol Membr Biol 2005, 22(1–2):51-61.

Condemine, G., Dorel, C., Hugouvieux-Cotte-Pattat, N., & Robert-Baudouy, J. (1992). Some of the out genes involved in the secretion of pectate lyases in *Erwinia chrysanthemi* are regulated by kdgR. Molecular microbiology, 6(21), 3199-3211.

Condemine, G., & Robert-Baudouy, J. (1995). Synthesis and secretion of *Erwinia chrysanthemi* virulence factors are coregulated. MPMI-Molecular Plant Microbe Interactions, 8(4), 632-636.

Condemine, G., Castillo, A., Passeri, F., & Enard, C. (1999). The PecT repressor coregulates synthesis of exopolysaccharides and virulence factors in *Erwinia chrysanthemi*. Molecular plant-microbe interactions, 12(1), 45-52.

Coulthurst, S. J., & Palmer, T. (2008). A new way out: protein localization on the bacterial cell surface via Tat and a novel Type II secretion system. Molecular microbiology, 69(6), 1331-1335.

Crépin A, Barbey C, Beury-Cirou A, Hélias V, Taupin L, Reverchon S, Nasser W, Faure D, Dufour A, Orange N, Feuilloley M, Heurlier K, Burini JF, Latour X. (2012) Quorum sensing signaling molecules produced by reference and emerging soft-rot bacteria (*Dickeya* and *Pectobacterium* spp.). PLoS One.7(4):e35176.

Cui, Y., Chatterjee, A., & Chatterjee, A. K. (2001). Effects of the two-component system comprising GacA and GacS of *Erwinia carotovora* subsp. *carotovora* on the production of global regulatory rsmB RNA, extracellular enzymes, and harpin Ecc. Molecular plant-microbe interactions, 14(4), 516-526.

Cuppels D., Kelman A. (1974) Evaluation of selective media for isolation of soft-rot bacteria from soil and plant tissue. Phytopathology, 64, 468–475.

Czajkowski R, de Boer WJ, VelvisH, van derWolf JM, 2010. Systemic colonization of potato plants by soil-borne,GFP-tagged strain of *Dickeya* sp. biovar 3. Phytopathology, 100, 134–42.

Czajkowski R, PerombelonMCM, van Veen JA, van derWolf JM, 2011. Control of blackleg and tuber soft rot of potato caused by Pectobacterium and *Dickeya* species: a review. Plant Pathology 60, 999–1013.

Czajkowski R., de Boer W.J., van der Zouwen P.S., Kastelein P., Jafra S., de Haan E. G., van den Bovenkamp G. W., & van der Wolf J. M. (2012) Virulence of '*Dickeya solani'* and *Dickeya dianthicola* biovar-1 and -7 strains on potato (Solanum tuberosum). Plant Pathology Doi: 10.1111/j.1365-3059.2012.02664.x

Czajkowski, R., Pérombelon, M. C. M., Jafra, S., Lojkowska, E., Potrykus, M., van der Wolf, J. M., & Sledz, W. (2015). Detection, identification and differentiation of *Pectobacterium* and *Dickeya* species causing potato blackleg and tuber soft rot: a review. *Annals of Applied Biology*, *166*(1), 18-38.

Dahler, G. S., Barras, F., & Keen, N. T. (1990). Cloning of genes encoding extracellular metalloproteases from *Erwinia chrysanthemi* EC16. Journal of bacteriology, 172(10), 5803-5815.

Darrasse, A., Priou, S., Kotoujansky, A., & Bertheau, Y. (1994). PCR and restriction fragment length polymorphism of a pel gene as a tool to identify *Erwinia carotovora* in relation to potato diseases. Applied and Environmental Microbiology, 60(5), 1437-1443.

Degefu Y., Potrykus M., Golanowska M., Virtanen E., Lojkowska E. (2013) A new clade of *Dickeya* spp. plays a major role in potato blackleg outbreaks in North Finland. Annals of Applied Biology, 162, 231–241

Dellagi A, Rigault M, Segond D, Roux C, Kraepiel Y, Cellier F, Briat JF, Gaymard F, Expert D (2005) Siderophore-mediated upregulation of Arabidopsis ferritin expression in response to *Erwinia chrysanthemi* infection. Plant J 43:262–272

Delepelaire P,Wandersman C. 1989. Protease secretion by *Erwinia chrysanthemi*. Proteases B and C are synthesized and secreted as zymogens without a signal peptide. J. Biol. Chem. 264(15):9083–89

Delepelaire, P., & Wandersman, C. (1990). Protein secretion in gram-negative bacteria. The extracellular metalloprotease B from *Erwinia chrysanthemi* contains a C-terminal secretion signal analogous to that of *Escherichia coli* alpha-hemolysin. Journal of Biological Chemistry, 265(28), 17118-17125.

Delepelaire P,WandersmanC. 1991. Characterization, localization and transmembrane organization of the three proteins PrtD, PrtE and PrtF necessary for protease secretion by the gram-negative bacterium *Erwinia chrysanthemi*. Mol. Microbiol. 5(10):2427–34

Dickey, R. S. (1979). *Erwinia chrysanthemi*: a comparative study of phenotypic properties of strains from several hosts and other *Erwinia* species. Phytopathology, 69, 324– 329.

Dutilh, B. E., Snel, B., Ettema, T. J., & Huynen, M. A. (2008). Signature genes as a phylogenomic tool. Molecular biology and evolution, 25(8), 1659-1667.

Effantin G, Rivasseau C, GromovaM, Bligny R, Hugouvieux-Cotte-PattatN. 2011. Massive production of butanediol during plant infection by phytopathogenic bacteria of the genera *Dickeya* and *Pectobacterium*. Mol. Microbiol. 82:988–97

Egel DS, Graham JH, Stall RE, 1991. Genomic relatedness of *Xanthomonas campestris* strains causing disease of citrus. Applied and Environmental Microbiology 57, 2724 – 30.

Elphinstone J., (1987) Soft Rot and Blackleg of Potato, Technical Information Bulletin 21, International Potato Center

Enard C, Diolez A, Expert D (1988) Systemic virulence of *Erwinia chrysanthemi* 3937 requires a functional iron assimilation system. J Bacteriol 170:2419–2426

English G, Byron O,Cianfanelli F, R. Prescott A., Coulthurst S. (2014). Biochemical analysis of TssK, a core component of the bacterial Type VI secretion system, reveals distinct oligomeric states of TssK and identifies a TssK-TssFG subcomplex. Biochemical Journal, 461(2), 291-304.

Expert D, Toussaint A (1985) Bacteriocin-resistant mutants of *Erwinia chrysanthemi*: possible involvement of iron acquisition in phytopathogenicity. J Bacteriol 163:221–227

Franza, T., & Expert, D. (2013). Role of iron homeostasis in the virulence of phytopathogenic bacteria: an 'a la carte'menu. Molecular plant pathology, 14(4), 429-438.

Franza T, Expert D (2010) Iron uptake in soft rot Erwinia. In: Cornelis P, Andrews SC (eds) Iron uptake and homeostasis in microorganisms. Caister Academic Press, Norwich, pp 101–115

Franza T,MaheB, ExpertD. (2005). Erwinia chrysanthemi requires a second iron transport routedependent on the siderophore achromobactin for extracellular growth and plant infection. Mol. Microbiol. 55:261–75

Franza T, Michaud-Soret I, Piquerel P, Expert D. (2002). Coupling of iron assimilation and pectinolysis in *Erwinia chrysanthemi* 3937. Mol. Plant-Microbe Interact. 15:1181–91

Franza T, Sauvage C, Expert D. (1999). Iron regulation and pathogenicity in *Erwinia chrysanthemi* 3937: role of the Fur repressor protein. Mol. Plant-Microbe Interact. 12:119–28

Fritsch, M. J., Trunk, K., Diniz, J. A., Guo, M., Trost, M. and Coulthurst, S. J. (2013) Proteomic identification of novel secreted antibacterial toxins of the type VI secretion system. Mol. Cell. Proteomics 12, 2735–2749

Galardini, M., Biondi, E. G., Bazzicalupo, M., & Mengoni, A. (2011). CONTIGuator: a bacterial genomes finishing tool for structural insights on draft genomes. Source code for biology and medicine, 6(11).

Galardini, M., Mengoni, A., Biondi, E. G., Semeraro, R., Florio, A., Bazzicalupo, M., Mocali, S. (2014). DuctApe: a suite for the analysis and correlation of genomic and OmniLog™ phenotype microarray data. Genomics, 103(1), 1-10.

Gao, B. & Gupta, R. S. (2012). Phylogenetic framework and molecular signatures for the main clades of the phylum Actino- bacteria. Microbiol Mol Biol Rev 76, 66–112.

Gardan L, Gouy C, Christen R, Samson R (2003) Elevation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrosepticum* sp. nov., *Pectobacterium betavasculorum* sp. nov. and *Pectobacterium wasabiae* sp. nov. Int J Syst Evol Microbiol 53: 381–391

Garlant L, Koskinen P, Rouhiainen L, Laine P, Paulin L, Auvinen P, Holm L, Pirhonen M. 2013. Genome sequence of *Dickeya solani*, a new soft rot pathogen of potato, suggests its emergence may be related to a novel combination of non-ribosomal peptide/polyketide synthetase clusters. Diversity 5:824–842. doi:10.3390/d5040824.

Ghigo, J. M., & Wandersman, C. (1992). Cloning, nucleotide sequence and characterization of the gene encoding the *Erwinia chrysanthemi* B374 PrtA metalloprotease: a third metalloprotease secreted via a C-terminal secretion signal. Molecular and General Genetics MGG, 236(1), 135-144.

Glasner JD, Marquez-Villavicencio M, Kim H-S, Jahn CE, Ma B, et al. 2008. Niche-specificity and the variable fraction of the *Pectobacterium* pan-genome. Mol. Plant-Microbe Interact. 21:1549–60

Glasner JD, Yang CH, Reverchon S, Hugouvieux-Cotte-Pattat N, Condemine G, Bohin JP, Van Gijsegem F, Yang S, Franza T, Expert D, Plunkett G 3rd, San Francisco MJ, Charkowski AO, Py B, Bell K, Rauscher L, Rodriguez-Palenzuela P, Toussaint A, Holeva MC, He SY, Douet V, Boccara M, Blanco C, Toth I, Anderson BD, Biehl BS, Mau B, Flynn SM, Barras F, Lindeberg M, Birch PR, Tsuyumu S, Shi X, Hibbing M, Yap MN, Carpentier M, Dassa E, Umehara M, Kim JF, Rusch M, Soni P, Mayhew GF, Fouts DE, Gill SR, Blattner FR, Keen NT, Perna NT. 2011 Genome sequence of the plant-pathogenic bacterium *Dickeya dadantii* 3937. Journal of Bacteriology 193:2076-2077.

Golanowska M, Galardini M, Bazzicalupo M, Hugouvieux-Cotte-Pattat N, Mengoni A, Potrykus M, Slawiak M, Lojkowska E. 2015. Draft genome sequence of a highly virulent strain of the plant pathogen *Dickeya solani*, IFB0099. Genome Announc 3(2):e00109-15. doi:10.1128/genomeA.00109-15.

Guiseppi, A., J.-L Aymeric, B. Cami, F. Barras, and N. Creuzet.1991. Sequence analysis of the cellulase-encoding celY gene of *Erwinia chrysanthemi*: a possible case of interspecies gene transfer.Gene 106:109-114.

Gupta, R. S. (2010). Applications of conserved indels for under- standing microbial phylogeny. In Molecular Phylogeny of Microor- ganisms, pp. 135–150. Edited by A. Oren & R. T. Papke. Norwich: Caister Academic Press.

Grenier, A. M., Duport, G., Pagès, S., Condemine, G., Rahbé, Y. (2006). The phytopathogen *Dickeya dadantii* (*Erwinia chrysanthemi* 3937) is a pathogen of the pea aphid. Applied and Environmental Microbiology, 72: 1956-1965.

Grijpstra J, Arenas J, Rutten L,Tommassen J. 2013. Autotransporter secretion: varying on a theme. Res. Microbiol. 164(6):562–82

Grothues D, Rudolph K, 1991. Macrorestriction analysis of plant pathogenic *Pseudomonas* species and pathovars. FEMS Microbiology Letters79, 83 – 8

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98.

Harshey, R. M. 2003. Bacterial motility on a surface: Many ways to a common goal. Annu. Rev. Microbiol. 57:249-273.

Hassan, S., Shevchik, V. E., Robert, X., & Hugouvieux-Cotte-Pattat, N. (2013). PelN is a new pectate lyase of *Dickeya dadantii* with unusual characteristics. Journal of Bacteriology, 195: 2197-2206.

Hauben L, Moore ERB, Vauterin L, Steenackers M, Mergaert J, et al. 1998. Phylogenetic position of phytopathogens within the Enterobacteriaceae. Syst. Appl.Microbiol. 21:384–97

Hélias V., Hamon P., Huchet E., van derWolf J.M., Andrivon D. (2012) Two new effective semiselective crystal violet pectate media for isolation of *Pectobacterium* and *Dickeya*. Plant Pathology, 61, 339–345.

Hérault, E., Reverchon, S., Nasser, W. (2014). Role of the LysR-type transcriptional regulator PecT and DNA supercoiling in the thermoregulation of pelgenes, the major virulence factors in *Dickeya dadantii*. Environmental Microbiology 16: 734-745.

Heilbronn, J., & Lyon, G. D. (1990). The ineffectuality of potato protease inhibitor on the extracellular protease from *Erwinia carotovora* subsp. *carotovora*. Journal of Applied Bacteriology, 69(1), 25-29.

Holeva MC, Bell KS, Hyman LJ, Avrova AO, Whisson SC, Birch PRJ, Toth IK, (2004) Use of a pooled transposon mutation grid to demonstrate roles in disease development for *Erwinia carotovora* subsp. *atroseptica* putative type III secreted effector (DspE/A) and helper (HrpN) proteins. Mol Plant Microbe Interact, 17:943-950.

Hommais, F., Oger-Desfeux, C., Van Gijsegem, F., Castang, S., Ligori, S., Expert, D., Nasser, W., Reverchon, S. (2008). PecS is a global regulator of the symptomatic phase in the phytopathogenic bacterium *Erwinia chrysanthemi* 3937. Journal of Bacteriology 190: 7508-7522.

Hugouvieux-Cotte-Pattat, N., & Robert-Baudouy, J. (1989). Isolation of *Erwinia chrysanthemi* mutants altered in pectinolytic enzyme production. Molecular microbiology, 3(11), 1587-1597.

Hugouvieux-Cotte-Pattat, N., G. Condemine, W. Nasser, & S. Reverchon.(1996). Regulation of pectinolysis in *Erwinia chrysanthemi*. Annual Review of Microbiology50: 213-257.

Hugouvieux-Cotte-Pattat, N., Condemine, G., & Shevchik, V. E. (2014). Bacterial pectate lyases, structural and functional diversity. Environmental microbiology reports, 6(5), 427-440.

Hunter, S. B., Vauterin, P., Lambert-Fair, M. A., Van Duyne, M. S., Kubota, K., Graves, L., Wrigley D., Barret T., Ribot, E. (2005). Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. Journal of Clinical Microbiology, 43(3), 1045-1050.

Hyman L.J., Toth I.K., Pérombelon M.C.M. (1998) Isolation and identification. In Methods for the Detection and Quantification of *Erwinia carotovora* subsp. atroseptica on Potato. Laboratory Manual, Scottish Crop Research Insti- tute Annual Report, Dundee, Scotland, The United King- dom, pp. 64–71.

Hyytiäinen, H., Montesano, M., & Palva, E. T. (2001). Global regulators ExpA (GacA) and KdgR modulate extracellular enzyme gene expression through the RsmA-rsmB system in *Erwinia carotovora* subsp. *carotovora*. Molecular plant-microbe interactions, 14(8), 931-938.

Jafra, S., Figura, I., Hugouvieux-Cotte-Pattat, N., & Lojkowska, E. (1999). Expression of *Erwinia chrysanthemi* pectinase genes pelI, pelL, and pelZ during infection of potato tubers. Molecular plant-microbe interactions, 12(10), 845-851.

Jahn, C. E., Willis, D. K., & Charkowski, A. O. (2008). The flagellar sigma factor fliA is required for *Dickeya dadantii* virulence. Molecular plant-microbe interactions, 21(11), 1431-1442.

Ji, J., Hugouvieux-Cotte-Pattat, N., Robert – Baudouy, J. (1987). Use of Mu-lac insertions to study the secretion of pectate lyases by *Erwinia chrysanthemi*. Journal of general microbiology, 133: 793-802.

Kazemi-Pour N, Condemine G, Hugouvieux-Cotte-Pattat N. 2004. The secretome of the plant pathogenic bacterium *Erwinia chrysanthemi*. Proteomics 4(10):3177–86

Kelemu S, Collmer A. 1993. *Erwinia chrysanthemi* EC16 produces a sec- ond set of plant-inducible pectate lyase isoenzymes. Appl. Environ. Microbiol. 59:1756–61

Khayi, S., Mondy, S., Beury-Cirou, A., Moumni, M., Hélias, V., Faure, D. (2014). Genome sequence of the emerging plant pathogen *Dickeya solani* strain RNS 08.23. 3.1 A. Genome announcements, 2: 1270-13.

Kim, H. S., Ma, B., Perna, N. T., & Charkowski, A. O. (2009). Phylogeny and virulence of naturally occurring type III secretion system-deficient *Pectobacterium* strains. Applied and Environmental Microbiology, 75(13), 4539-4549.

Kim H-S, Thammarat P, Lommel SA, Hogan CS, Charkowski AO. 2011. *Pectobacterium carotovorum* elicits plant cell death with DspE/F, but does not suppress callose or induce expression of plant genes early in plant-microbe interactions. Mol. Plant-Microbe Interact. 24:773–86

Kotoujansky, A., Lemattre, M., & Boistard, P. (1982). Utilization of a thermosensitive episome bearing transposon TN10 to isolate Hfr donor strains of *Erwinia carotovora* subsp. *chrysanthemi*. Journal of bacteriology, 150(1), 122-131.

Kotoujansky A. 1987. Molecular genetics of pathogenesis by soft-rot erwinias. Annu. Rev. Phytopathol. 25:405–30

Koskiniemi, S., Lamoureux, J.G., Nikolakakis, K.C.,T'Kint de Roodenbeke, C., Kaplan, M.D., Low, D.A., and Hayes, C.S. (2013) Rhs proteins from diverse bacteria mediate intercellular competition. Proc Natl Acad Sci U S A. 110: 7032–7037.

Kretschmer, P. J., & Egan, J. B. (1975). Genetic map of the Staphylococcal bacteriophage phi11. Journal of virology, 16(3), 642-651.

Kyöstiö, S.R.M., Cramer, C.L. and Lacy, G.H. (1991) *Erwinia carotovora* subsp. *carotovora* extracellular protease: characterisation and nucleotide sequence of the gene. J. Bacteriol. 173, 6537– 6546

Lapierre P, Gogarten JP. (2009) Estimating the size of the bacterial pan-genome. Trends Genet. 25(3):107-10.

Laurila, J., Ahola, V., Lehtinen, A., Joutsjoki, T., Hannukkala, A., Rahkonen, A., & Pirhonen, M. (2008). Characterization of *Dickeya* strains isolated from potato and river water samples in Finland. European Journal of Plant Pathology 122, 213-225.

Laurilla, J., Hannukkala, A., Nykyri, J., Pasanen, M., Hélias, V., Garlant, L., Pirhonen, M. (2010). Symptoms and yield reduction caused by *Dickeya* spp. strains isolated from potato and river water in Finland. European Journal of Plant Pathology 126: 249-262.

Lebeau A, Reverchon S, Gaubert S, Kraepiel Y, Simond-Cˆote E, et al. 2008. TheGacA global regulator is required for the appropriate expression of *Erwinia chrysanthemi* 3937 pathogenicity genes during plant infection. Environ.Microbiol. 10:545–59

Lee Y.A., Yu C.P. (2005) A differential medium for the isola- tion and rapid identification of a plant soft rot pathogen, *Erwinia chrysanthemi*. Journal of Microbiological Methods, 64, 200–206

Lee Y.A., Chen K.P., Hsu Y.W. (2006) Characterization of *Erwinia chrysanthemi*, the soft-rot pathogen of white- flowered calla lily, based on pathogenicity and PCR-RFLP and PFGE analyses. Plant Pathology, 55, 530–536.

Lelliott, R. A., & Dickey, R. S. (1984). Genus VII. Erwinia. Bergey's manual of systematic bacteriology, 1, 469-476.

Leo JC, Grin I, Linke D. 2012. Type V secretion: mechanism(s) of autotransport through the bacterial outer membrane. Philos. Trans. R. Soc. Lond. B 367(1592):1088–101

Lerat, E., Daubin, V., Ochman, H., & Moran, N. A. (2005). Evolutionary origins of genomic repertoires in bacteria. PLoS Biol, 3(5), e130.

Letoffe S, Delepelaire P,Wandersman C. 1989. Characterization of a protein inhibitor of extracellular proteases produced by *Erwinia chrysanthemi*. Mol. Microbiol. 3(1):79–86

Letoffe, S., Delepelaire, P., & Wandersman, C. (1990). Protease secretion by *Erwinia chrysanthemi*: the specific secretion functions are analogous to those of *Escherichia coli* alpha-haemolysin. The EMBO journal, 9(5), 1375.

Linhartova I, Bumba L, Maˇs´ın J, Basler M, Osiˇcka R, et al. 2010. RTX proteins: a highly diverse family secreted by a common mechanism. FEMS Microbiol. Rev. 34(6):1076–112

Liu H, Coulthurst SJ, Pritchard L, Hedley PE, Ravensdale M, et al. (2008) Quorum sensing coordinates brute force and stealth modes of infection in the plant pathogen *Pectobacterium atrosepticum*. PloS Pathogens 20: e1000093.

Lojkowska E, Dorel C, Reignault P, Hugouvieux-Cotte-Pattat N, Robert- Baudouy J. 1993. Use ofGUSfusions to study the expression of *Erwinia chrysanthemi* pectinase genes during infection of potato tubers. Mol. Plant-Microbe Interact. 6:488–94

Lojkowska E, Masclaux C, Boccara M, Robert-Baudouy J, Hugouvieux-Cotte- Pattat N. 1995. Characterization of the pelL gene encoding a novel pectate lyase of *Erwinia chrysanthemi* 3937. Mol. Microbiol. 16:1183–95

Lojkowska E, Potrykus M, SławiakM, 2010.Molecular characterization and pathogenicity of *Dickeya* strains isolated recently from potato plants in Poland. In: Proceedings of the 12th International Conference on Plant Pathogenic Bacteria, 7–11 June 2010, StDenis, Reunion, 158.

Maas Geesteranus HP, 1972. Natrot en zwartbenigheid bij aardappelen. Bedrijfsontwikkeling 3, 941–5.

Ma B, Hibbing ME, Kim H-S, Reedy RM, Yedidia I, et al. (2007) Host range and molecular phylogenies of the soft rot enterobacterial genera *Pectobacterium* and *Dickeya.* Phytopathology 97: 1150–1163.

Mansfield J., Genin S., Magori S., Citovsky V., Sriariyanum M., Ronald P., Dow M., Verdier V., Beer S.V., Machado M.A., Toth I., Salmond G., Foster G.D. (2012) Top 10 plant pathogenic bacteria in molecular plant pathology. Molecular Plant Pathology, 13, 614–629.

Marits R, Koiv V, Laasik E, Mae A. 1999. Isolation of an extracellular protease gene of *Erwinia carotovora* subsp. *carotovora* strain SCC3193 by transposon mutagenesis and the role of protease in phytopathogenic- ity. Microbiology 145:1959–66

Marits R, TshuikinaM, PirhonenM, Laasik E, Mae A. 2002. Regulation of the expression of prtW::gusA fusions in *Erwinia carotovora* subsp. *carotovora*. Microbiology 148:835–42

Mattinen L, Somervuo P, Nykyri J, Nissinen R, Kouvonen P, Corthals G, Auvinen P, Aittamaa M, Valkonen JP, & Pirhonen M. (2008) Microarray profiling of host-extract-induced genes and characterization of the type VI secretion cluster in the potato pathogen *Pectobacterium atrosepticum*. Microbiology 154:2387-2396.\

McMillan, G. P., Barrett, A. M., & Perombelon, M. C. M. (1994). An isoelectric focusing study of the effect of methyl-esterified pectic substances on the production of extracellular pectin isoenzymes by soft rot *Erwinia* spp. Journal of applied bacteriology, 77(2), 175-184.

Mhedbi-Hajri N, Malfatti P, P´edron J, Gaubert S, Reverchon S, Van Gijsegem F. (2011). PecS is an important player in the regulatory network governing the coordinated expression of virulence genes during the interaction between *Dickeya dadantii* 3937 and plants. Environ.Microbiol. 13:2901–14

Miller, J.H. (1972) Experiments in Molecular Genetics. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Moran, F., S. Nasuno, and M. P. Starr. 1968. Extracellular and intracellular polygalacturonic acid trans-eliminases of *Erwinia carotovora*. Arch. Biochem. Biophys. 123:298-306.

Mulholland, V., Hinton, J. C., Sidebotham, J., Toth, I. K., Hyman, L. J., Perombelon, M., Salmond, G. P. (1993). A pleiotropic reduced virulence (Rvi−) mutant of *Erwinia carotovora* subspecies atroseptica is defective in flagella assembly proteins that are conserved in plant and animal bacterial pathogens. Molecular microbiology, 9(2), 343-356.

Nachin, L., & Barras, F. (2000). External pH: an environmental signal that helps to rationalize pel gene duplication in *Erwinia chrysanthemi*. Molecular plant-microbe interactions, 13(8), 882-886.

Naushad, H. S., Lee, B., & Gupta, R. S. (2014). Conserved signature indels and signature proteins as novel tools for understanding microbial phylogeny and systematics: identification of molecular signatures that are specific for the phytopathogenic genera *Dickeya*, *Pectobacterium* and *Brenneria*. International journal of systematic and evolutionary microbiology, 64(Pt 2), 366-383.

Nasser, W., Reverchon, S. & Robert-Baudouy, J. 1992. Purification and functional characterization of the KdgR protein, a major repressor of pectinolysis genes of *Erwinia chrysanthemi*. Molecular Microbiology 6:257-265.

Nasser, W., Reverchon, S., Condemine, G., & Robert-Baudouy, J. (1994). Specific interactions of *Erwinia chrysanthemi* KdgR repressor with different operators of genes involved in pectinolysis. Journal of molecular biology, 236(2), 427-440.

Nasser, W., Robert-Baudouy, J., & Reverchon, S. (1997). Antagonistic effect of CRP and KdgR in the transcription control of the *Erwinia chrysanthemi* pectinolysis genes. Molecular microbiology, 26(5), 1071-1082.

Nasser W, Bouillant ML, Salmond G, Reverchon S. (1998) Characterization of the *Erwinia chrysanthemi*expI-expR locus directing the synthesis of two N-acyl-homoserine lactone signal molecules. Molecular Microbiology Sep;29(6):1391-405.

Nasser, W., & Reverchon, S. (2002). H-NS-dependent activation of pectate lyases synthesis in the phytopathogenic bacterium *Erwinia chrysanthemi* is mediated by the PecT repressor. Molecular microbiology, 43(3), 733-748.

Nasser, W., Reverchon, S., Vedel, R., & Boccara, M. (2005). PecS and PecT coregulate the synthesis of HrpN and pectate lyases, two virulence determinants in *Erwinia chrysanthemi* 3937. Molecular plant-microbe interactions, 18(11), 1205-1214.

Nasser, W., Dorel, C., Wawrzyniak, J., Van Gijsegem, F., Groleau, M. C., Déziel, E., & Reverchon, S. (2013). Vfm a new quorum sensing system controls the virulence of *Dickeya dadantii*. Environmental microbiology, 15(3), 865-880.

Ngadze E., Brady C.L., Coutinho T., Van Der Waals J.E. (2012) Pectinolytic bacteria associated with potato soft rot and blackleg in South Africa and Zimbabwe. European Journal of Plant Pathology, 134, 533–549

Nguyen, H. A., Kaneko, J., & Kamio, Y. (2002). Temperature-dependent production of carotovoricin Er and pectin lyase in phytopathogenic *Erwinia carotovora* subsp. *carotovora* Er. Bioscience, biotechnology, and biochemistry, 66(2), 444-447.

Nykyri J, Niemi O, Koskinen P, Nokso-Koivisto J, Pasanen M, et al. (2012). Revised phylogeny and novel horizontally acquired virulence determinants of the model soft rot phytopathogen *Pectobacterium wasabiae* SCC3193. PLoS Pathog. 8(11):e1003013

Nykyri, J. (2013). Virulence of Soft-Rot Enterobacteria Affecting Potato. Department of Agricultural Sciences Publications.

Nykyri, J., Fang, X., Dorati, F., Bakr, R., Pasanen, M., Niemi, O., Pirhonen, M. (2014). Evidence that nematodes may vector the soft rot-causing enterobacterial phytopathogens. Plant Pathology, 63(4), 747-757.

Nomura, K., W. Nasser, H. Kawagishi, & S. Tsuyumu. (1998). The pir gene of *Erwinia chrysanthemi* EC16 regulates hyperinduction of pectate lyase virulence genes in response to plant signals.Proceedings of the National Academy of Sciences U. S. A.95: 14034-14039.

Palacio-Bielsa A., Cambra M.A., LópezM.M. (2006) Charac- terisation of potato isolates of *Dickeya chrysanthemi* in Spain by a microtitre system for biovar determination. Annals of Applied Biology, 148, 157–164

Parkinson, N., DeVos, P., Pirhonen, M., Elphinstone, J. (2014). *Dickeya aquatica* sp. nov., isolated from waterways. Internationa Journal of Systematic and Evolutionary Microbiology 64: 2264-6.

ParkinsonN, Pritchard L, Bryant R, Toth I, Elphinstone J., (2015). Epidemiology of *Dickeya dianthicola* and *Dickeya solani* in ornamental hosts and potato studied using variable number tandem repeat analysis. European Journal of Plant 141:523. DOI: 10.1007/s10658-014-0523-5

Pédron, J., Mondy, S., des Essarts, Y. R., Van Gijsegem, F., Faure, D. (2014). Genomic and metabolic comparison with *Dickeya dadantii* 3937 reveals the emerging *Dickeya solani* potato pathogen to display distinctive metabolic activities and T5SS/T6SS-related toxin repertoire. BMC genomics 15: 283.

Pérez-Mendoza, D., Coulthurst, S. J., Humphris, S., Campbell, E., Welch, M., Toth, I. K., & Salmond, G. P. (2011). A multi-repeat adhesin of the phytopathogen, *Pectobacterium atrosepticum*, is secreted by a Type I pathway and is subject to complex regulation involving a non-canonical diguanylate cyclase. Molecular microbiology, 82(3), 719-733.

Perombelon, MCM, Kelman A. 1980. Ecology of the soft rot erwinias. Annu. Rev. Phytopathol. 18:361–87

Perombelon, MCM,Lumb VM, Zutra D, 1987. Pathogenicity of soft roterwinias to potato plants inScotlandand Israel. Journal of Applied Microbiology 63, 73–84.

Perombelon, M. C.M.,&Salmond,G. P. C. (1995). Bacterial soft rots. In U. S. Singh, R. P. Singh & K. Kohmoto (Eds.), Pathogenesis and Host Specificity in Plant Diseases, Vol. 1

Perombelon, MCM, (2002). Potato diseases caused by soft rot erwinias: an overview of pathogenesis. Plant Pathology 51,1– 12.

Perombelon, M. C. M., & Van der Wolf, J. M. (2002). Methods for the detection and quantification of Erwinia carotovora subsp. atroseptica (*Pectobacterium carotovorum* subsp. *atrosepticum*) on potatoes: a laboratory manual. Dundee, Scotland: Scottish Crop Research Institute Occasional Publication, (10).

Pidwirny, M. (2006). "Climate Classification and Climatic Regions of the World". Fundamentals of Physical Geography, 2nd Edition.

Pitman, A. R., Harrow, S. A., & Visnovsky, S. B. (2009). Genetic characterisation of *Pectobacterium wasabiae* causing soft rot disease of potato in New Zealand. European Journal of Plant Pathology, 126(3), 423-435.

Pirhonen, M., Saarilahti, H., Karlsson, M. B., & Palva, E. T. (1991). Identification of pathogenicity determinants of *Erwinia carotovora* subsp. *carotovora* by transposon mutagenesis. Mol. Plant-Microbe Interact, 4(3), 276-83.

Pissavin, C., Robert-Baudouy, J., Hugouvieux-Cotte-Pattat, N. (1996). Regulation of *pelZ,* a gene of the *pelB-pelC* cluster encoding a new pectate lyase of *Erwinia chrysanthemi* 3937. Journal of Bacteriology, 178: 7187-7196.

Poole, S.J., Diner, E.J., Aoki, S.K., Braaten, B.A., t' Kint de Roodenbeke, C., Low, D.A. & Hayes, C.S. 2011. Identification of functional toxin/immunity genes linked to contact-dependent

growth inhibition (CDI) and rearrangement hotspot (Rhs) systems. PLoS Genetics 7:e1002217.

Potrykus, M., Golanowska, M., Hugouvieux-Cotte-Pattat, N., Lojkowska, E. (2014a). Regulators involved in *Dickeya solani* virulence, genetic conservation, and functional variability. Molecular Plant-Microbe Interactions 27: 700-711.

Potrykus, M., Sledz, W., Golanowska, M., Slawiak, M., Binek, A., Motyka, A., Zoledowska, S., Czajkowski, R.,Lojkowska, E. (2014b). Simultaneous detection of major blackleg and soft rot bacterial pathogens in potato by multiplex polymerase chain reaction. Annals of Applied Biology, 165: 474-487.

Potrykus M., Golanowska M., Sledz W., Zoledowska S., Motyka A., Kolodziejska A., Butrymowicz J., lojkowska E., (2015, in press) Biodiversity of *Dickeya* spp. isolated from potato plants and water sources in temperate climate

Praillet, T., Nasser, W., Robert-Baudouy, J., & Reverchon, S. (1996). Purification and functional characterization of PecS, a regulator of virulence-factor synthesis in *Erwinia chrysanthemi*. Molecular microbiology, 20(2), 391-402.

Praillet, T., Reverchon, S., & Nasser, W. (1997). Mutual control of the PecS/PecM couple, two proteins regulating virulence-factor synthesis in *Erwinia chrysanthemi*. Molecular microbiology, 24(4), 803-814.

Pritchard, L., Humphris, S., Baeyen, S., Maes, M., Van Vaerenbergh, J., Elphinstone, J., Saddler, G., Toth, I. (2013a). Draft genome sequences of four *Dickeya dianthicola* and four *Dickeya solani* strains. Genome announcements, 1(4).

Pritchard, L., S. Humphris, G.S. Saddler, J.G. Elphinstone, M. Pirhonen, and I.K. Toth. (2013b). Draft genome sequences of 17 isolates of the plant pathogenic bacterium *Dickeya*. Genome Announcements1:e00978–13

Py, B., Isabelle, B. G., Haiech, J., Chippaux, M., Barras, F. (1991). Cellulase EGZ of *Erwinia chrysanthemi:* structural organization and importance of His98 and Glu133 residues for catalysis. Protein Engineering, 4: 325-333.Résibois, A., Pato, M., Higgins, P., and Toussaint, A. (1984) Replication ofbacteriophage mu and its mini-mu derivatives. Adv Exp Med Biol 179: 69-76.

Py, B., Barras, F., Harris, S., Robson, N., & Salmond, G. P. C. (1998). Extracellular enzymes and their role in *Erwinia* virulence. In P. Williams, J. Ketley, & G. Salmond (Eds.), Bacterial Pathogenesis: Methods in Microbiology (vol. 27, (pp. 157–168)). London, UK: Academic Press Ltd

Resibois, A., Colet, M., Faelen, M., Schoonejans, E., & Toussaint, A. (1984). ΦEC2, a new generalized transducing phage of *Erwinia chrysanthemi*. Virology, 137(1), 102-112.

Reverchon, S., Van Gijsegem, F., Rouve, M., Kotoujansky, A., Robert-Baudouy, J. (1986). Organization of a pectate lyase gene family in *Erwinia chrysanthemi*. Gene, 49: 215-224.

Reverchon, S., Nasser, W., Robert-Baudouy, J (1991). Characterization of kdgR, a gene of *Erwinia chrysanthemi* that regulates pectin degradation. Molecular Microbiology 5: 2203-2216.

Reverchon, S., Nasser, W., & Robert-Baudouy, J. (1994). pecS: a locus controlling pectinase, cellulase and blue pigment production in *Erwinia chrysanthemi*. Molecular microbiology, 11(6), 1127-1139.

Reverchon S., Bouillant M.L., Salmond G., Nasser W. (1998). Integration of the quorum-sensing system in the regulatory networks controlling virulence factor synthesis in *Erwinia chrysanthemi*. Molecular Microbiology 29: 1407-1418.

Reverchon, S., Rouanet, C., Expert, D., & Nasser, W. (2002). Characterization of indigoidine biosynthetic genes in *Erwinia chrysanthemi* and role of this blue pigment in pathogenicity. Journal of bacteriology, 184(3), 654-665.

Reverchon, S., Nasser, W. (2013). *Dickeya* ecology, environment sensing and regulation of virulence programme. Environmental Microbiology Reports 5: 622-636.

Ribot EM, Fitzgerald C, Kubota K, Swaminathan B, Barrett TJ, 2001. Rapid pulsed-field gel electrophoresis protocol for subtyping of Campylobacter jejuni. Journal of Clinical Microbiology 39, 1889–94

Richter, M., & Rosselló-Móra, R. (2009). Shifting the genomic gold standard for the prokaryotic species definition. Proceedings of the National Academy of Sciences, 106(45), 19126-19131.

Rojas CM, Ham JH, Deng W-L, Doyle JJ, Collmer A. 2002. HecA, a member of a class of adhesins produced by diverse pathogenic bacteria, contributes to the attachment, aggregation, epidermal cell killing, and virulence phenotypes of *Erwinia chrysanthemi* EC16 on *Nicotiana clevelandii* seedlings. Proc. Natl. Acad. Sci. USA 99(20):13142–47

Rouanet, C., Reverchon, S., Rodionov, D. A., & Nasser, W. (2004). Definition of a consensus DNA-binding site for PecS, a global regulator of virulence gene expression in *Erwinia chrysanthemi* and identification of new members of the PecS regulon. Journal of Biological Chemistry, 279(29), 30158-30167.

Roy, C., Kester, H., Visser, J., Shevchik, V., Hugouvieux-Cotte-Pattat, N., Robert-Baudouy, J., & Benen, J. (1999). Modes of action of five different endopectate lyases from *Erwinia chrysanthemi* 3937. Journal of bacteriology, 181(12), 3705-3709.

RPD - Report on plant disease, Department of Crop Sciences, University of Illinois at Urbana-Champaign, RPD No. 943 July 1990

Ruhe ZC, Low DA, Hayes CS. 2013. Bacterial contact-dependent growth inhibition. Trends Microbiol. 21(5):230–37

Russell, A. B., Singh, P., Brittnacher, M., Bui, N. K., Hood, R. D., Carl, M. A., Agnello, D. M., Schwarz, S., Goodlett, D. R., Vollmer, W. and Mougous, J. D. (2012) A widespread bacterial type VI secretion effector superfamily identified using a heuristic approach. Cell Host Microbe 11, 538–549

Sahl, J. W., Caporaso, J. G., Rasko, D. A., & Keim, P. (2014). The large-scale blast score ratio (LS-BSR) pipeline: a method to rapidly compare genetic content between bacterial genomes. PeerJ, 2, e332.

Salmond, G. P. C. 1994. Secretion of extracellular virulence factors by plant pathogenic bacteria. Annu. Rev. Phytopathol. 32:181–200.

Samson, R., Poutier, F., Sailly, M., Jouan, B. (1987). Caractérisation des *Erwinia chrysanthemi* isolées de Solanum tuberosum et d'autres plantes-hôtes selon les biovars et sérogroupes. EPPO Bulletin 17: 11-16.

Samson, R., Legendre, J. B., Christen, R., Saux, M. F. L., Achouak, W. & Gardan, L. (2005). Transfer of *Pectobacterium chrysanthemi* (Burkholder et al. 1953) Brenner et al. 1973 and *Brenneria paradisiaca* to the genus *Dickeya* gen. nov. As *Dickeya chrysanthemi* comb. nov. and *Dickeya paradisiaca* comb. nov. And delineation of four novel species, *Dickeya dadantii* sp. nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zeae* sp. International Journal of Systematic and Evolutionary Microbiology55, 1415-1427.

Sauvage, C., & Expert, D. (1994). Differential regulation by iron of *Erwinia chrysanthemi* pectate lyases: pathogenicity of iron transport regulatory (cbr) mutants. Molecular Plant Microbe Interactions, 7, 71-71.

Schwyn B, Neilands JB, 1987. Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry 160, 47–56.

Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. doi:10.1093/bioinformatics/btu153.

Sepulchre, J.A., Reverchon, S., and Nasser, W. (2007) Modeling the onset of virulence in a pectinolytic bacterium. J Theor Biol 244: 239-257.

Schauer K, Rodionov DA, de Reuse H. 2008. New substrates for TonB-dependent transport: Do we only see the "tip of the iceberg?" Trends Biochem. Sci. 33:330–38

Shevchik, V. E., Robert-Baudouy, J., Hugouvieux-Cotte-Pattat, N. (1997). Pectate lyase PelI of *Erwinia chrysanthemi* 3937 belongs to a new family. Journal of Bacteriology, 179: 7321-7330.

Shih, Y. L., Harris, S. J., Borner, G., Rivet, M. M., & Salmond, G. P. (1999). The hexY genes of *Erwinia carotovora* ssp. *carotovora* and ssp. *atroseptica* encode novel proteins that regulate virulence and motility co-ordinately. Environmental microbiology, 1(6), 535-547.

Sławiak M., Łojkowska E., & van der Wolf (2009a) First report of bacterial soft rot on potato caused by *Dickeya* spp. (syn. *Erwinia chrysanthemi*) in Poland. Plant Pathology 58: 794.

Sławiak M., van Beckhoven J.R.C.M., Speksnijder A.G.C.L., Czajkowski R., Grabe G., & van der Wolf J.M (2009b) Biochemical and genetic analysis reveal a new clade of biovar 3 *Dickeya* spp. strains isolated from potato in Europe. European Journal of Plant Pathology 125: 245-261.

Surgey, N., Robert-Baudouy, J., & Condemine, G. (1996). The *Erwinia chrysanthemi* pecT gene regulates pectinase gene expression. Journal of bacteriology, 178(6), 1593-1599.

Tarasova, N., Gorshkov, V., Petrova, O., & Gogolev, Y. (2013). Potato signal molecules that activate pectate lyase synthesis in *Pectobacterium atrosepticum* SCRI1043. World Journal of Microbiology and Biotechnology, 29(7), 1189-1196.

Tardy, F., Nasser, W., Robert-Baudouy, J., Hugouvieux-Cotte-Pattat, N. (1997). Comparative analysis of the five major *Erwinia chrysanthemi* pectate lyases: enzyme characteristics and potential inhibitors. Journal of bacteriology, 179: 2503-2511.

Tettelin, H. et al. (2005) Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae*: implications for the microbial ‘‘pan-genome’’. Proc. Natl. Acad. Sci. U. S. A. 102, 13950–13955

Thomson, N. R., Thomas, J. D., & Salmond, G. P. (1999). 12 Virulence Determinants in the Bacterial Phytopathogen *Erwinia*. Genetic Methods for Diverse Prokaryotes, 29, 347.

Toth, I. K., K. S. Bell, M. C. Holeva, and P. R. Birch. (2003). Soft rot erwiniae: from genes to genomes.Molecular Plant Pathology4:17-30.

Toth, I. K., Pritchard, L., & Birch, P. R. (2006). Comparative genomics reveals what makes an enterobacterial plant pathogen. Annu. Rev. Phytopathol., 44, 305-336.

Toth I. K., van der Wolf J. M., Saddler G., Lojkowska E., Hélias V., Pirhonen M., Tsror (Lahkim) L., & Elphinstone J.G. (2011) *Dickeya* species: an emerging problem for potato production in Europe. Plant Pathology 60: 385–399.

Tseng, T. T., Tyler, B. M., & Setubal, J. C. (2009). Protein secretion systems in bacterial-host associations, and their description in the Gene Ontology. BMC microbiology, 9

Tsor (Lahkim) L, Erlich O, Lebiush S, Hazanovsky M, Zig U (2009) Assessment of recent outbreaks of *Dickeya* sp. (syn. *Erwinia chrysanthemi*) slow wilt in potato crops in Israel. Eur J Plant Pathol 123: 311–320.

Tsror (Lahkim) L., Ben-Daniel B., Chalupowicz L., van der Wolf J., Lebiush S., Erlich O., Dror O., Barel V., Nijhuis E., Manulis-Sasson S. (2013) Characterization of *Dickeya* strains isolated from potato grown under hot-climate con- ditions. Plant Pathology, 62, 1097–1105.

Van Gijsegem F, Woldarczyk A, Cornu A, Reverchon S, & Hugouvieux-Cotte-Pattat N.(2008). Analysis of the LacI family regulators of *Erwinia chrysanthemi* 3937, involvement in the bacterial phytopathogenicity. Molecular Plant-Microbe Interactions21: 1471-1481.

Van der Wolf J.M., de Haas B.H., van Hoof R., de Haan, E.G., van den Bovenkamp G.W. (2014a) Development and evaluation of Taqman assays for the differentiation of *Dickeya*(sub)species.European Journal of Plant Pathology,138, 695–709

van der Wolf J.M., Nijhuis E.H., Kowalewska M.J., Saddler G.S., Parkinson N., Elphinstone J.G., Pritchard L., Toth I.K., Lojkowska E., Potrykus M.,Waleron M., de Vos P., Cleen- werck I., Pirhonen M., Garlant L., Hélias V., Pothier J.F., Pflüger V., Duffy B., Tsror L., Manulis S. (2014b) *Dickeya solani* sp. nov., a pectinolytic plant pathogenic bacterium isolated from potato (Solanum tuberosum). International Jour- nal of Systematic and Evolutionary Microbiology, 64, 768–774. DOI:10.1099/ijs.0.052944-0

Van Vaerenbergh, J., Baeyen, S., De Vos, P. & Maes, M. (2012) Sequence diversity in the *Dickeya fliC* gene: phylogeny of the *Dickeya* Genus and TaqMan (R) PCR for '*D. solani'*, new Biovar 3 variant on potato in Europe, PLoS, One 7.

Versalovic, J., Koeuth, T., & Lupski, R. (1991). Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Research, 19, 6823–6831. doi:10.1093/nar/19.24.6823

Versalovic J., Schneider M., De Bruijn F.J., Lupski J.R. (1994) Genomic fingerprinting of bacteria using repeti- tive sequence-based polymerase chain reaction. Methods in Molecular and Cellular Biology, 5, 25–40.

Waldee E. L 1945 Comparative studies of some peritrichous -phytopathogenic bacteria. Iowa State Coll. Jci., 19,435-484.

Waleron M., Waleron K., Podhajska A.J., Łojkowska E. (2002a) Genotyping of bacteria belonging to the former *Erwinia* genus by PCR-RFLP analysis of a *recA* gene treat- ment. Microbiology, 148, 583–595.

Waleron, M., Waleron, K., & Lojkowska, E. (2002b). Genotypic characterisation of the *Erwinia* genus by PCR-RFLP analysis of rpoS Gene. Plant protection science-prague-, 38, 288-290.

Waleron M., Waleron K., Lojkowska E. (2013) Occurrence of *Pectobacterium wasabiae* in potato field samples. European Journal of Plant Pathology, 137, 149–158

Winslow, C. E. A., Broadhurst, J., Buchanan R. E., Krumwiede, C., Rogers, L.A., Smith, G. H. (1917). The families and genera of the bacteria. Preliminary report of the Committee of the Society of American Bacteriologists: Characterization and Classification of Bacterial Types. Journal of Bacteriology 2: 505-566.

Whitney, J. C., Chou, S., Russell, A. B., Biboy, J., Gardiner, T. E., Ferrin, M. A., Brittnacher, M., Vollmer, W. and Mougous, J. D. (2013) Identification, structure, and function of a novel type VI secretion peptidoglycan glycoside hydrolase effector–immunity pair. J. Biol. Chem. 288, 26616–26624

Wood, P. J. (1980). Specificity in the interaction of direct dyes with polysaccharides. Carbohydrate research, 85: 271-287.

Yao, J., & Allen, C. (2006). Chemotaxis is required for virulence and competitive fitness of the bacterial wilt pathogen Ralstonia solanacearum. Journal of bacteriology, 188(10), 3697-3708.

Zhang Y, Geider K, (1997). Differentiation of *Erwinia amylovora* strains by pulsed-field gel electrophoresis. Applied and Environmental Microbiology 63, 4421–6.

ZhangY, BakDD,Heid H, Geider K. (1999). Molecular characterization of a protease secreted by *Erwinia amylovora*. J. Mol. Biol. 289(5):1239–51

INSA INSTITUT NATIONAL DES SCIENCES APPLIQUÉES LYON

FOLIO ADMINISTRATIF

THÈSE SOUTENUE DEVANT L'INSTITUT NATIONAL DES SCIENCES APPLIQUÉES DE LYON

NOM : GOLANOWSKA DATE de SOUTENANCE : 25 sept 2015

Prénoms : Malgorzata

TITRE :
Characterization of *Dickeya solani* strains and identification of bacterial and plant signals involved in induction of virulence

NATURE : Doctorat Numéro d'ordre : 2015ISAL0087

Ecole doctorale : E2M2

Spécialité : Microbiology

RESUME :
Dickeya solani is a species consisting of newly emerged plant pathogenic bacteria that cause blackleg and soft rot diseases. They are responsible for great damages to potato plantations in most of European countries. *D. solani* strains produce a wide range of plant cell-wall degrading enzymes which are the main virulence factors.
The aims of the study were: 1) phenotypic and genotypic characterizations of the *D. solani* strains isolated in countries with different climatic conditions: Poland, Finland and Israel, 2) study of the potato tuber extract influence on the expression of a few selected *D. solani* genes : *pelD, pelL*, *tssK*, *lfaA*,3) comparative genomics of ten *D. solani* strains, performed on 4 genomes sequenced for this study and 6 genome sequences available in the GenBank databases.
All the genetic studies proved that *D. solani* strains form a very homogenous group. On the other hand, the phenotypic analysis showed some variability among strains from different climatic conditions. The observed variations in the phenotypic traits can results from a different regulation of the expression of the genes encoding virulence factors which are influenced by temperature, pH, iron deprivation, oxygen and nitrogen availability, as well as by the presence of plant compounds

MOTS-CLÉS :
plant pathogenic bacteria, plant cell-wall degrading enzymes, soft-rot, comparative genomics, plant signals

Laboratoire (s) de recherche :
- Microbiology, Adaptation & Pathogenesis (MAP), UMR5240, INSA de Lyon
- Plant Protection and Biotechnology (LPP&B), Intercollegiate Faculty of Biotechnology and Medical University of Gdansk

Directeurs de thèse:
COTTE-PATTAT Nicole, Research Director CNRS (INSA de Lyon, France)
LOJKOWSKA Ewa, Professor (Gdansk, Poland)

Président de jury :
OBUCHOWSKI Michał, Professor and vice dean of the Faculty of Biotechnology (Gdansk, Poland)

Composition du jury :
COTTE-PATTAT Nicole, Research Director, CNRS (INSA de Lyon, France)
JAWORSKI Adam, Professor University of Lodz (Poland)
LOJKOWSKA Ewa, Professor (Gdansk, Poland)
MAZUR Andrzej, Professor UMCS Lublin (Poland)
OBUCHOWSKI Michał, Professor and vice dean of the Faculty of Biotechnology (Gdansk, Poland)
REVERCHON Sylvie, Associate Professor INSA de Lyon (France),
VAN GIJSEGEM Frederique, Research Director INRA, AgroParisTech (France)